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A PYTHIUM SEEDLING BLIGHT AND ROOT ROT OF DENT CORN [1]

By HELEN JOHANN, *Assistant Pathologist*, and JAMES R. HOLBERT, *Agronomist, Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*, and JAMES G. DICKSON, *Professor of Plant Pathology, University of Wisconsin, and Agent, Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

During the course of investigations of the corn rots caused by *Diplodia zeae*, *Gibberella saubinetii*, and *Fusarium moniliforme* certain phases of the root-rot problem made it increasingly apparent that in all probability other organisms than these named are to be considered important causes of the diseases.

The cortical rots produced by the three fungi previously mentioned are confined chiefly to the coleorhiza and the mesocotyl and spread from them into the cortex of the primary and adventitious roots of the corn seedling. The development of the cortical rotting is in marked contrast to a distinct type of root rotting that begins at the tips of the rootlets and reaches the mesocotyl only in the late stages of seedling blight.

In 1923, when a species of Pythium was isolated from the roots of corn plants grown in the field at Madison, Wis., and from seedlings that had blighted in unsterilized soil in the greenhouse, the present investigation was started to determine to what extent, if any, Pythium is a factor in the corn root-rot and seedling-blight problems. The study also included a comparison of the symptoms produced under controlled conditions with those observed in rotting rootlets in the field. Since 1923 experiments have been conducted in the greenhouse and in the field at Madison, Wis., and in the field at Bloomington, Ill.

PYTHIUM INJURY

The severity of the Pythium injury depends upon environmental conditions. Germination may be prevented at low temperatures (12° to 16° C.), particularly in wet soils, by the rapid rotting of the embryo during the period of imbibition of moisture. A water-soaked appearance gives evidence of the presence of the fungus within the

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embryo. Discoloration of the water-soaked tissue follows the entrance of secondary organisms. Under conditions less favorable for the development of the disease, a soft rot of the seedling root system may develop, resulting in the gradual reduction of the functioning roots. In such plants subsequent invasion of the mesocotyl occurs and seedling blight results. Or, a mild root rot may occur, not severe enough to kill the seedling, yet resulting in a reduction in size and vigor, due to the decreased root surface and perhaps to the effect of toxins produced in the invaded cortical areas.

The influence of such stunting upon the final yield has been demonstrated by Holbert and his coworkers (*7*).[3] They state that, under field conditions, a direct correlation exists between early height and vigor and yield of corn plants.

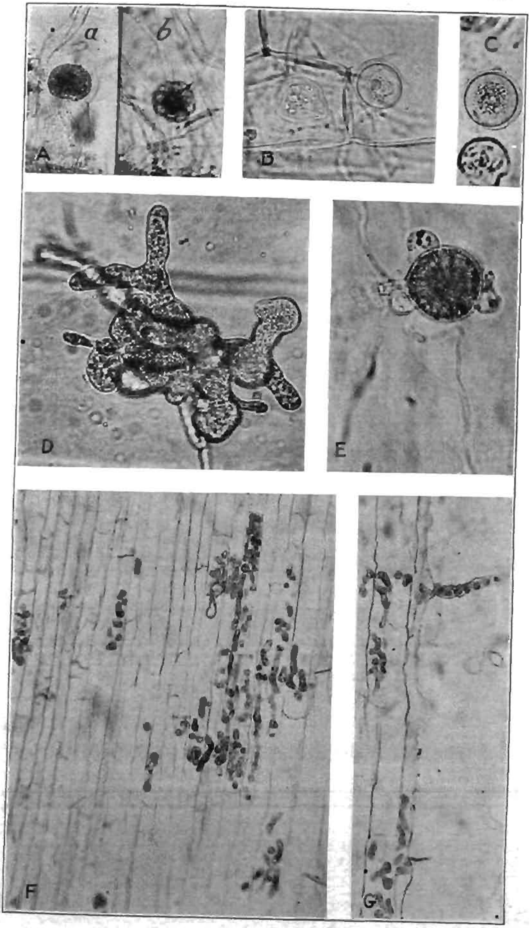
The greenhouse and field experiments have shown that Pythium injury is unlike that caused by *Diplodia zeae* and *Gibberella saubinetii* in that Pythium does not attack the mesocotyl of the corn seedling except occasionally in severe cases of invasion. Although the roots may be severely rotted, the mesocotyl usually remains clean. The experiments have shown that infection occurs near the tips of the rootlets and travels upward, much as described by Harter for the Pythium rootlet rot of sweet potatoes (*6*). The fungus produces a soft rot first in the root cortex and later in the vascular elements. There is no marked discoloration of these invaded tissues before secondary organisms make their entrance into the water-soaked tissue. Lobulate sporangia occur in the cortex, usually near the surface, and frequently appear in the root hairs. (Pl. 1, F, G.) It is exceedingly difficult to trace the course of the fungus through the root, because the granular protoplasm moves from the older mycelium into the tip as hyphal invasion progresses. The position of the fungus in the cortex is evident chiefly by the presence of the lobulate sporangia, as the thin-walled hyphae are practically invisible. Later conidia and oospores may be found within the vascular tissue as well as in the cortex of the root. The fruiting bodies ordinarily appear in greater numbers in rootlets grown at low soil temperatures.

In order to secure satisfactory material for study, it was necessary to use great care in the removal of infected plants from the soil in the greenhouse and in the field, so as to prevent breaking and loss of the rotted rootlets. Their recovery from large plants in the field was particularly difficult and uncertain.

[3] Reference is made by number (italic) to "Literature cited," p. 464.

EXPLANATORY LEGEND FOR PLATE 1

A.—Zoosporangia from corn rootlets: *a*, before and, *b*, after zoospore formation. × 200.
B.—Oospore within the cortex of a corn seedling root, grown on a plate of soil-decoction agar in an ordinary refrigerator. × 400.
C.—Oospore formed on soil-decoction agar near the rootlet of a sterile corn seedling. Oospores were not found on the agar far from the roots of the seedlings. Grown in refrigerator.
D.—Lobulate-sporangium-grown-on water agar at room temperature. Culture 8 days old. Highly magnified.
E.—Oogonium and antheridia from a 10-day-old culture grown in the refrigerator on grated-carrot agar. × 550.
F.—Lobulate sporangia within the cortex of a corn seedling grown in wet soil at 16° C. × 200.
G.—Lobulate sporangium in a root hair of a corn seedling grown in wet soil at 16° C. × 200.



(For explanation of legend see p. 444)

CAUSAL ORGANISM

CULTURAL CHARACTERISTICS

Isolations were made by plating diseased tissues on water agar after carefully washing them in sterile water. A disinfectant was not used, as Pythium is sensitive to mercuric chloride and acids. The fungus grows rapidly and usually within two or three days grows away from any bacteria or other fungi that may be present, and therefore can be transferred readily. On water agar or on soil-decoction agar the mycelial growth is so delicate that it is scarcely visible unless examined by transmitted light. Oatmeal, potato-dextrose, and corn-meal agars favor a more dense, white mycelial growth. In water at room temperature complex masses of lobulate sporangia, called presporangia by Edson *(4)*, form within a day or two along hyphae growing out of infected tissue. They may also be obtained within a week along hyphae on soft agars as poor in food materials as water agar or soil-decoction agar. (Pl. 1, D.) On these agars no conidia, oogonia, or antheridia have been found. They are numerous, however, at low temperatures in agar in which there is a plentiful supply of bits of plant tissue, such as grated carrots or string beans. (Pl. 1, E.) The conidia and oogonia are very similar in appearance during the early stages of their development. Later, under certain conditions, the conidia send out one or more germ tubes and the oogonia have one or more club-shaped antheridia applied to them. On the vegetable agars used, fertilization evidently is not of common occurrence, as comparatively few oospores are found. Likewise, in these media, typical lobulate sporangia are seldom observed, although thickenings of portions of the hyphae occur.

All three types of fruiting bodies have been obtained, however, within sterile roots of germinating corn seedlings. (Pl. 1.) For the most part they have been obtained from corn roots growing on inoculated soil-decoction agar in Petri dishes at relatively low temperatures (10° to 16° C.). Zoospores have been obtained within a few hours from corn roots containing lobulate sporangia by placing the roots in water in a Petri dish kept at room temperature. (Pl. 1, A.)

TEMPERATURE RELATIONS

The temperature range of this fungus in culture is wide. Two separate series of duplicate plates of potato-dextrose agar were inoculated in the center with squares, of as nearly equal size as possible, of a young agar culture and were incubated for three days at different temperatures ranging from 4° to 42° C. Mycelium, visible with a microscope, developed within 48 hours at 4° and at 40°, but an exposure of 72 hours at 42° killed the fungus. Maximum growth on this medium, as indicated by the diameters of the colonies, occurred between 30° and 36°.

The temperature at which the culture is grown, as well as the medium upon which it is grown, affects fruiting. Numerous lobulate sporangia appear within a few days on a favorable medium when grown at temperatures ranging from 20° to 30° C. These bodies are formed more slowly and in lesser numbers at the ordinary refrigerator temperatures (10° to 16°). On the other hand, oospore formation is favored by storage at these temperatures.

Subramaniam *(10)*, who described *Pythium butleri* from India, found somewhat the same general conditions affecting its fruiting.

He states: "The development of sporangia and oogonia follows no regular sequence. When cultures are kept in a cool incubator at 21° C., oospores appear first and later on sporangia. At 30° C. sporangia appear first."

TAXONOMY

As stated by the writers in an earlier publication (*8*), this Pythium on corn is somewhat closely related to *Pythium butleri* Subr. on sugar cane, as described by Carpenter (*1*), who considered this species synonymous with *Rheosporangium aphanidermatum* Edson (*4*). Since then Fitzpatrick (*5*) has transferred this species to the genus Pythium, making the binomial *P. aphanidermatum* (Eds.) Fitzpatrick, inasmuch as Edson's work antedated that of Subramaniam.

Drechsler (*3*) reported *Pythium aphanidermatum* as the causal organism of cottony leak of cucumbers. He added that "a species not yet identified, provided with lobulate sporangia and hence closely related to but not identical with *P. aphanidermatum*, which was isolated from diseased corn roots, has shown no evidence of pathogenicity on cucumber fruit." The writers also have found cucumber fruit not an especially favorable medium for the growth of their corn Pythium. The fungus has proved mildly pathogenic to cucumbers, however, in a few wound inoculations.

Recently Valleau and his associates (*12*) report finding numerous oospores of the Pythium type in rotting corn roots. They suggest that "corn root rot, other than seedling blights known to be caused by certain seed-borne organisms, is caused by a fungus similar to the fungus causing the cane root rot."

The corn Pythium resembles the fungus reported by Carpenter as causing a root rot of sugar cane, in that it produces lobulate sporangia within the cortex of the roots and in culture. These are comparable to those described for the sugar-cane fungus. Likewise the measurements of the smooth oogonia and oospores correspond rather closely (Table 1) to those given by Carpenter.

TABLE 1.—*A comparison of certain characters of Rheosporangium aphanidermatum (4), Pythium butleri (10), the cane Pythium (1), and P. arrhenomanes*

[Measurements in microns]

Fungus	Zoospores	Oogonia		Oospores	
		Range	Average	Range	Average
R. aphanidermatum	12×7.5	22–27			17–19
P. butleri	8–12×6–8	24–33	26	13.5–25.3	21
Cane Pythium	10–12×7.5–9.5	[a] 24–35	29	21–28	24
		[b] 20–26		18–24	25
		[c] 21–30		20–25	22
P. arrhenomanes		24–35	29.9	21–28	24.4

[a] Culture. [b] Cane root from field. [c] Cane root in water culture.

Drechsler, who is making a special study of this group, has kindly examined cultures from the writers' material and finds that, while the fungus somewhat resembles *Pythium aphanidermatum* and certain related species, it differs distinctly. In an article by Drechsler, now in press, the parasite is being described as *P. arrhenomanes*.[4]

[4] This paper by Drechsler is now published. DRECHSLER, C. PYTHIUM ARRHENOMANES N. SP., A PARASITE CAUSING MAIZE ROOT ROT. Phytopathology 18: 873–875. 1928

EXPERIMENTAL STUDIES

GREENHOUSE EXPERIMENTS

Experiments dealing with the effect of soil temperature and soil moisture on the occurrence of the disease were conducted in the Wisconsin soil-temperature control tanks in the greenhouses of the University of Wisconsin during the winters of 1923–24 and 1924–25. The temperatures used ranged from 12° to 32° C., at approximately 4° intervals. Experiments in the 32° tanks were discontinued during the second winter because of the scarcity of available tanks and because the checks and inoculated seedlings were equally poor when grown at such a high temperature under glass. Steam-sterilized soil was used throughout the experiments. Soil moisture was controlled by weighing the cans at intervals and adding the quantity of water necessary to maintain a constant weight. Different methods of watering were tried, but none was devised that could insure a uniform moisture distribution throughout the can, especially in the drier soils. When only one moisture was used in an experiment the soil was made up to a good growing moisture. When two moistures were employed the wet soil contained as much water as could be added and the soil screened and handled without packing—that is, about 70 per cent of saturation. The dry soils ranged from 45 to 55 per cent of saturation at the beginning of the experiments. In three series the inoculum was added to the soil some days previous to planting, and in the others small squares of water-agar or soil-decoction agar culture were placed near each seed at the time of planting. The control plants were grown in the sterilized soil without the addition of agar.

TABLE 2.—*Results of inoculating inbred strains of yellow dent corn with Pythium arrhenomanes at the time of planting in the greenhouse, Madison, Wis., 1923–1925*

Experiment No.	Date	Strain No.	Soil moisture	Inoculum used	Number of kernels germinating at— 12° C	16° C	20° C	24° C	28° C	Reduction in germination following inoculation	Number of plants standing at end of experiment at— 12° C	16° C	20° C	24° C	28° C	Reduction in stand following inoculation[a]	Mean length of tops at— 16° C	20° C	24° C	28° C	Reduction following inoculation	Mean dry weight of tops at— 16° C	20° C	24° C	28° C	Reduction following inoculation
										P. ct.						P. ct.	Cm.	Cm.	Cm.	Cm.	P. ct.	Gm.	Gm.	Gm.	Gm.	P. ct.
5	Oct. 10 to Dec. 12	Hy-826-1-5-1.	Wet	None	15	16	16	16	16	-----	11	14	16	13	16	-----	10.7	17.4	28.7	27.7	-----	0.535	0.954	2.565	2.715	-----
				Pythium	14	16	16	16	15	2.53	13	16	15	11	14	1.43	8.7	17.0	25.4	27.5	6.98	.262	.669	1.575	2.905	20.06
			Dry	None	8	14	15	13	15	-----	8	11	15	8	13	-----	7.5	13.8	16.7	15.1	-----	.280	.378	.275	.695	-----
				Pythium	6	13	16	14	15	1.54	6	11	16	10	14	[a]+3.64	4.7	10.7	13.4	17.0	13.75	.151	.232	.300	.700	15.05
		Hy-826-1-6-1.	Wet	None	12	12	15	16	14	-----	12	11	14	15	11	-----	8.5	14.1	22.3	25.8	-----	.446	.743	1.845	2.890	-----
				Pythium	15	12	15	15	16	[a]+5.80	8	4	14	14	13	15.87	5.1	12.9	22.0	21.9	12.45	.158	.804	1.945	1.725	21.81
			Dry	None	8	15	8	11	7	-----	8	[b]8	8	10	6	-----	6.0	9.9	12.7	11.7	-----	.164	.412	.427	.437	-----
				Pythium	12	11	10	10	11	[a]+10.20	11	11	10	10	11	[a]+32.50	5.2	7.9	16.2	12.8	[a]+4.47	.131	.243	.650	.342	5.14
7 and 8	Feb. 9 to Apr. 24[c] and	A-1-1-2-R-1-2-3.	Wet	None	16	16	14	16	16	-----	16	16	14	15	15	-----	15.7	24.2	38.5	30.5	-----	-----	-----	-----	-----	-----
				Pythium	0	0	15	14	15	43.59	0	0	14	14	15	43.42	0	13.6	33.4	29.3	29.94	-----	-----	-----	-----	-----
			Dry	None	15	14	15	13	16	-----	15	14	15	11	15	-----	12.9	21.6	38.0	26.2	-----	-----	-----	-----	-----	-----
				Pythium	0	14	16	16	16	15.07	0	14	16	14	16	14.29	9.6	16.1	31.5	27.0	14.69	-----	-----	-----	-----	-----
	Mar. 7 to Apr. 24[d]	A-1-1-4-1-8-1-5.	Wet	None	10	16	16	16	14	-----	10	16	16	16	14	-----	9.2	21.6	35.5	26.1	-----	-----	-----	-----	-----	-----
				Pythium	1	1	11	13	11	48.61	1	1	11	13	10	50.00	4.0	10.1	25.7	22.8	32.25	-----	-----	-----	-----	-----
			Dry	None	11	13	16	15	15	-----	11	13	16	14	15	-----	7.7	19.4	33.5	24.4	-----	-----	-----	-----	-----	-----
				Pythium	0	2	14	16	15	32.86	0	2	14	15	13	36.23	7.5	13.3	26.8	22.5	17.53	-----	-----	-----	-----	-----
9	May 4 to May 29	B-1-1-3-R-10-1-12.	Wet	None	-----	15	16	16	16	-----	-----	15	11	15	16	-----	10.9	22.3	40.5	50.2	-----	-----	.337	.720	.750	-----
				Pythium	-----	15	15	16	16	1.59	-----	13	15	16	16	[a]+5.26	7.0	17.8	32.6	40.7	20.82	-----	.206	.318	.437	46.82
			Dry	None	-----	16	16	16	16	-----	-----	16	16	16	14	-----	10.7	21.8	38.9	45.8	-----	-----	.312	.637	.715	-----
				Pythium	-----	16	16	16	16	0	-----	14	16	16	16	0	5.4	20.1	36.4	45.0	8.79	-----	.243	.456	.543	25.36
		A-1-1-1-R-4-2-4.	Wet	None	-----	16	16	16	14	-----	-----	16	16	15	13	-----	11.8	18.2	35.2	47.2	-----	-----	.250	.660	.669	-----
				Pythium	-----	13	15	16	16	3.23	-----	13	15	16	16	0	7.7	15.3	31.0	42.8	13.88	-----	.180	.450	.493	28.88
			Dry	None	-----	16	16	16	16	-----	-----	16	16	15	16	-----	10.4	19.1	34.0	45.1	-----	-----	.238	.526	.556	-----
				Pythium	-----	16	16	16	16	0	-----	15	15	16	16	1.59	8.0	16.7	34.2	42.1	7.00	-----	.206	.512	.518	6.36
		G-146	Wet	None	-----	[b]16	16	16	16	-----	-----	15	16	15	14	-----	22.03	32.5	45.5	43.71	-----	.093	.206	.333	.213	-----
				Pythium	-----	12	16	[b]16	16	6.25	-----	5	15	11	13	26.67	17.80	24.4	44.5	52.07	3.46	.060	.140	.318	.361	[a]+4.02
			Dry	None	-----	15	15	16	16	-----	-----	15	15	16	16	-----	17.40	26.26	41.4	44.30	-----	.073	.106	.218	.231	-----
				Pythium	-----	14	15	16	16	1.61	-----	10	15	16	15	9.68	15.35	26.20	37.2	43.80	5.26	.050	.125	.187	.233	5.25
[illegible]	Oct. 24 to Dec. 10	A-1-1-2-R-1-2-3.	Wet	None	-----	16	15	16	[b]16	-----	-----	12	15	15	15	-----	22.83	34.26	50.66	56.50	-----	.077	.213	.506	.600	-----
				Pythium	-----	10	16	[b]16	16	7.94	-----	3	13	13	15	22.81	12.66	29.61	51.50	53.80	10.16	.016	.161	.484	.546	13.54
			Dry	None	-----	[b]16	16	16	16	-----	-----	12	15	15	15	-----	18.41	32.73	50.73	48.60	-----	.066	.166	.368	.366	-----
				Pythium	-----	[b]16	15	16	15	3.13	-----	15	15	14	14	[a]+1.75	16.20	35.00	45.79	44.90	5.70	.065	.180	.342	.357	2.28
Per cent reduction following inoculation					49.5	25.2	1.66	0.82	[a]+.84	-----	57.1	33.2	2.14	2.2	[a]+1.34	-----	33.43	17.9	9.81	4.03	-----	48.5	21.5	17.0	15.5	-----

[a] + denotes increase.

[b] 1 can with 4 plants discarded.

[c] 12° C.

[d] 16° to [illegible]

TABLE 3.—*Summary of the results of inoculating inbred strains of yellow dent corn with Pythium arrhenomanes at the time of planting in the greenhouse, Madison, Wis., 1923–1925*

Temperature (°C.)	Kernels germinating			Plants standing at end of experiment			Mean length of tops			Mean dry weight of tops		
	Control (number)	Inoculated (number)	Reduction following inoculation (per cent)	Control (number)	Inoculated (number)	Reduction following inoculation (per cent)	Control (cm.)	Inoculated (cm.)	Reduction following inoculation (per cent)	Control (gram)	Inoculated (gram)	Reduction following inoculation (per cent)
12	95	48	49.5	91	39	57.1	-----	-----	-----	-----	-----	-----
16	242	181	25.2	220	147	33.2	12.7	8.4	33.4	0.216	0.111	48.5
20	241	237	1.7	234	229	2.1	21.8	17.9	17.9	.359	.282	21.5
24	244	242	.8	224	219	2.2	35.2	31.7	9.8	.756	.628	17.0
28	239	241	[a]+.8	224	227	[a]+1.3	35.6	34 1	4.0	.903	.763	15.5

[a] + denotes increase.

The data obtained in these experiments from the use of the inbred strains are given in Table 2, summarized in Table 3, and presented graphically in Figure 1. Those obtained from the use of open-pollinated yellow dent corn are given in Table 4 and shown graphically in Figure 2.

TABLE 4.—*Results of inoculating open-pollinated yellow dent corn with Pythium arrhenomanes at time of planting in the greenhouse, Madison, Wis., 1923–24*

Temperature (°C.)	Kernels germinating			Plants standing at end of experiment			Mean length of tops			Mean dry weight of tops		
	Control (number)	Inoculated (number)	Reduction following inoculation (per cent)	Control (number)	Inoculated (number)	Reduction following inoculation (per cent)	Control (cm.)	Inoculated (cm.)	Reduction following inoculation (per cent)	Control (gram)	Inoculated (gram)	Reduction following inoculation (per cent)
16	28	22	21.4	27	20	25.9	23.39	18	23.0	1.05	0.52	50.5
20	29	28	[a]3.4	25	27	[a][b]+8.0	29.50	26.23	[a]11.1	2.07	1.31	[a]36.7
24	28	29	[b]+3.6	27	28	[b]+3.7	29.66	24.86	16.2	2.12	1.32	37.7
28	30	30	0	24	24	0	27.30	24.29	11.0	1.93	1.34	30.6
32	29	30	[b]+3.4	19	20	[b]+5.3	23.46	24.20	[b]+3.2	1.12	1.17	[b]+4.5

[a] The drop in these percentages at 20° was due in some measure to the presence of an electric light hung directly above the 20° tank and kept lighted throughout the night.
[b] + denotes increase.

At the higher temperatures germination was not materially affected following inoculation with *Pythium arrhenomanes*. Soil temperatures as low as 12° C. caused an uneven stand in both the uninoculated and the inoculated series, indicating an extremely unfavorable condition for the corn seedlings. At 16° the reduction in germination following inoculation averaged 25.2 per cent in the inbred strains (Tables 2 and 3) and 21.4 per cent in the open-pollinated corn (Table 4). In the inbred strains the reduction ranged from zero in strains B–1–1–3–R–10–1–12 and A–1–1–1–R–4–2–4 to 100 per cent in strain A–1–1–2–R–1–2–3. (Fig. 3.) Kernels planted in wet soil usually were more severely attacked than those in dry soil. At 20°

the average reduction was only 1.66 per cent (Tables 2 and 3) and the greatest reduction was 31.3 per cent.

The lack of uniformity in germination occurring in the dry soil may be ascribed in part at least to the difficulty of securing an even distribution of moisture throughout the dry soil after watering began.

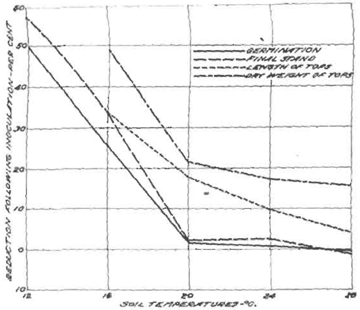


FIG. 1.—Reductions in germination of seed, in final stand, and in length and dry weight of tops of corn plants grown at different soil temperatures from inbred seed, inoculated with *Pythium arrhenomanes* at the time of planting. (Data in Table 3)

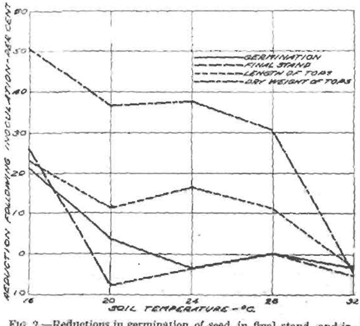


FIG. 2.—Reductions in germination of seed, in final stand, and in length and dry weight of tops of corn plants grown at different soil temperatures from open-pollinated seed, inoculated with *Pythium arrhenomanes* at the time of planting. (Data in Table 4)

Blighting of seedlings after emergence further reduced the stand, and low soil temperatures favored this manifestation of the disease.

A reduction in plant height and dry weight of tops followed inoculation with *Pythium arrhenomanes*. The highest percentage of reduction both in height and in dry weight of tops occurred at the lower temperatures. High soil moisture also tended to favor this manifesta-

tion of the disease. These plants showed no indication of the disease except the dwarfing of the aboveground parts. The tops were of good color and unwilted. Upon removal from the soil, however, the root systems were found to be smaller and in most cases partially destroyed. The root rot is not confined to those strains most susceptible to seedling blight. Strain B–1–1–3–R–10–1–12, with a good record for germination, was reduced in height and in dry weight of

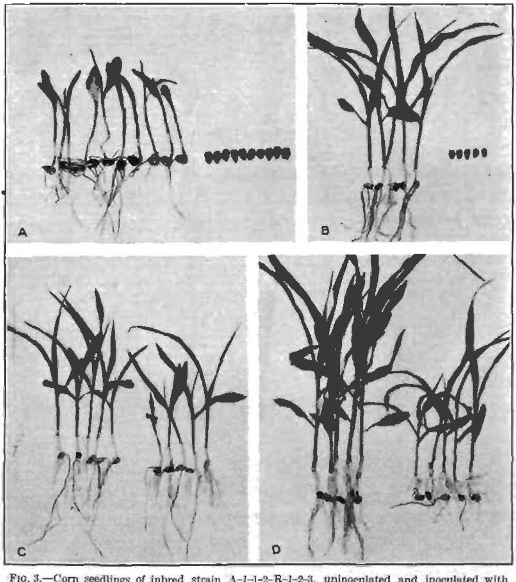


FIG. 3.—Corn seedlings of inbred strain A–1–1–2–R–1–2–3, uninoculated and inoculated with *Pythiumar rhenomanes* at time of planting, grown at different soil temperatures and in wet and dry soil. In each case the seedlings from uninoculated seed are grouped at the left and those from inoculated seed at the right. A, Grown in wet soil at 12° C.; B, grown in wet soil at 16°; C, grown in dry soil at 16°; D, grown in wet soil at 20°

tops following inoculation (fig. 4), although, as pointed out above, germination was not reduced materially. *P. arrhenomanes* was observed in or isolated from diseased roots at all temperatures.

FIELD EXPERIMENTS

In addition to the greenhouse inoculation experiments, field inoculations were made near Bloomington, Ill., in 1924, 1925, and 1926. In these field experiments the weather conditions were studied in connection with the development of the disease and the relative susceptibility of the different strains of corn.

Weather Conditions and Experiments in 1924

The spring of 1924 was cool, with a well-distributed rainfall during the period of seedling growth of the corn plants. The mean air

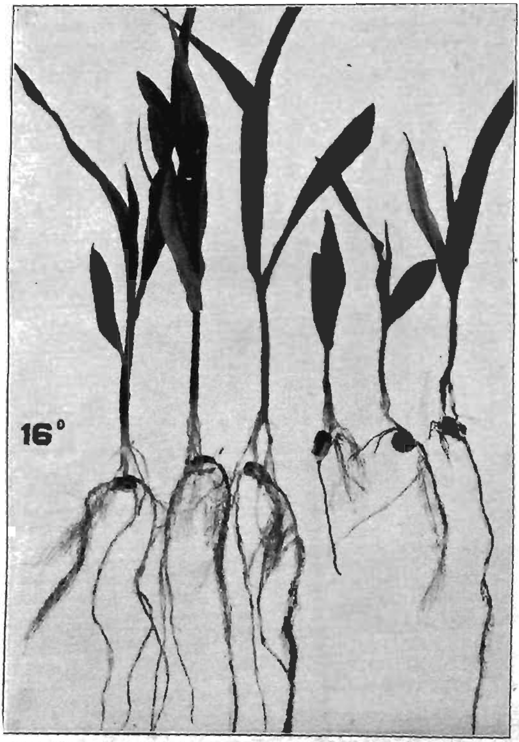


FIG. 4.—Corn seedlings of inbred strain B-1-1-3-R-10-1-12. The three at the left were uninoculated; the three at the right were inoculated with *Pythium arrhenomanes* at the time of planting. Grown in moderately wet soil at 16° C.

nperature at Bloomington, Ill., for the month of May was 6.4° below the normal (*11*). For June the departure from normal s −3.4. Precipitation in May was 3.2 inches, 1.36 inches below

the average. In June, however, a 2.5-inch rainfall on the 22d brought the total for the month to 7.81 inches, an excess of 4.2 inches. The field temperatures during the period of seedling development of the corn plant in 1924 (fig. 5) were comparable, in general, to the conditions in the low-temperature tanks in the greenhouse. The mean air and soil temperatures were obtained for each day by adding the maximum and minimum temperatures and dividing by 2.

Twenty-three inbred strains and one open-pollinated strain of yellow dent corn were used in the field experiments in 1924. The corn was checked 3½ feet apart with three kernels in each hill. Five rows of each strain were planted in each of two positions in the field. The first five hills of each row, which were left uninoculated, served as a control; the next five hills in each of the five rows were

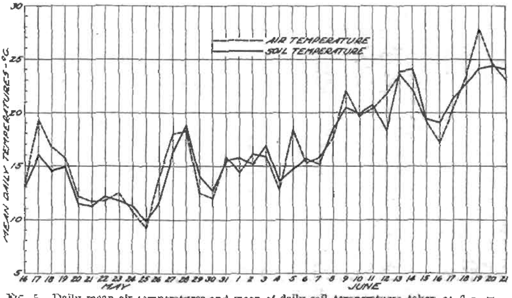


FIG. 5.—Daily mean air temperatures and mean of daily soil temperatures taken at 6 a. m. and 5.30 p. m. at Bloomington, Ill.

inoculated with sand cultures[5] of *Pythium arrhenomanes*. The following five hills served as a control. The succeeding five hills in each row were inoculated with conidial suspensions of *Gibberella saubinetii;* the next five hills were controls, after which the Pythium inoculation and controls were repeated. The results presented, therefore, represent a mean of the four 25-hill inoculated plots for each selfed line compared with the eight adjacent 25-hill control plots. The inoculum used for Pythium consisted of a small mass of mycelium growing in corn meal and white sand, which was placed in each hill just before the kernels were dropped into it. The control plots were planted without the addition of the sterilized sand and corn meal, as insufficient corn meal was used in the medium to affect materially the seedling growth of the control plants.

[5] The corn-meal-sand medium was prepared by washing 1 volume of quartz sand in distilled water, draining off the water but not drying the sand, and adding to this 1 volume of yellow corn meal and 1 to 1½ volumes of water. After a thorough stirring the mixture was steamed for 1 hour, stirred again, and allowed to cool. It was then broken into small pieces, placed in flasks, and autoclaved for 45 minutes at a pressure of 10 to 12 pounds on 2 successive days. Rapid growth of the fungus is obtained if the flasks are inoculated as soon as prepared. Cultures used in these experiments were usually about 10 days old.

[illegible] and inbred strains of yellow dent corn with *Pythium arrhenomanes* planted near Bloomington, Ill., May 16, 1924

Strains inoculated	Inoculum used	Number of replications	Mean field stand (per cent)	Mean yield per plant (pounds)	Effect of inoculation on plant yield: Increase (pounds)	Effect of inoculation on plant yield: Decrease (pounds)	Effect of inoculation on plant yield: Odds	Acre yield: Total (bushels)	Acre yield: Sound corn (bushels)	Effect on acre yield, Total: Increase (bushels)	Effect on acre yield, Total: Decrease (bushels)	Effect on acre yield, Total: Odds	Effect on acre yield, Sound corn: Increase (bushels)	Effect on acre yield, Sound corn: Decrease (bushels)	Effect on acre yield, Sound corn: Odds
Open-pollinated:															
Nearly disease-free seed (176A)	None	5	79.7	0.864				88.0	73.8						
	Pythium	5	64.0	.819		0.045	8:1	66.6	58.1		21.4	>9999:1		15.7	462:1
Diplodia-infected seed (176A)	None	5	48.5	.908				57.1	45.6						
	Pythium	5	34.9	.941	0.033		1:1	40.7	31.9		16.4	70:1		13.7	188:1
Inbred:															
A-1-1-1-R-4-2	None	4	54.6	.506				34.3	24.2						
	Pythium	4	38.7	.441		.065	4999:1	19.3	9.8		15.0	>9999:1		14.4	>9999:1
A-1-1-2-R-1-1-2	None	4	57.0	.443				30.2	25.5						
	Pythium	4	28.7	.500	.057		20:1	16.2	14.8		14.0	516:1		11.7	26:1
A-1-1-3-R-3-1	None	4	45.4	.357				22.3	17.4						
	Pythium	4	20.7	.474	.117		78:1	13.1	9.9		9.2	41:1		7.5	40:1
A-1-1-4-1-8-1	None	4	33.4	.490				20.9	15.3						
	Pythium	4	28.0	.476		.014	322:1	17.1	9.4		3.8	113:1		5.9	322:1
A-1-1-4-1-9-5	None	4	37.4	.324				14.6	8.6						
	Pythium	4	32.0	.375	.051		4:1	14.4	9.2		.2	1:1	0.6		10:1
B-1-1-1-1-8-1	None	4	50.7	.280				15.1	5.9						
	Pythium	4	58.7	.244		.036	55:1	15.3	6.4	0.2			.5		4:1
B-1-1-3-R-7-3	None	4	60.0	.563				40.4	24.9						
	Pythium	4	24.0	.514		.049	1666:1	14.8	8.0		25.6	>9999:1		16.9	1082:1
B-1-1-3-R-10-2	None	4	62.0	.526				43.1	32.8						
	Pythium	4	57.3	.523		.003	1:1	39.6	29.0		3.5	4:1		3.8	4:1
K-4-3-1	None	4	60.0	.400				37.0	27.0						
	Pythium	4	38.7	.578	.178		>9999:1	26.8	17.6		10.2	55:1		9.4	35:1

G-8-8-1	None	4	72.0	.502				42.8	33.8						
	Pythium	4	51.7	.384		.118	>9999:1	21.5	18.7		18.3	>9999:1		15.1	>9999:1
L-1-1-1-1	None	4	71.3	.676				47.3	41.7						
	Pythium	4	47.4	.548		.128	160:1	26.5	20.4		20.8	>9999:1		21.3	>9999:1
ID-3-1-1-1-1-1	None	1	72.0	.515				46.1	27.4						
	Pythium	1	40.0	.377		.138		18.6	9.1		27.5			18.3	
228-4-8-2-1	None	1	74.7	.434				36.1	19.9						
	Pythium	1	42.7	.463	.029			22.1	12.0		14.0			7.9	
W-2-1-3	None	1	72.0	.315				23.6	7.3						
	Pythium	1	53.3	.188		.127		11.6	2.7		15.0			4.6	
W-10-2-2	None	1	74.7	.625				59.2	47.3						
	Pythium	1	58.7	.541		.084		40.2	33.4		19.0			13.9	
M-3-3-1	None	1	65.3	.527				43.3	30.7						
	Pythium	1	53.3	.495		.032		33.2	17.6		10.1			13.1	
HY-826-1-5-1	None	4	72.7	.594				46.7	35.6						
	Pythium	4	60.0	.610	.016		3:1	39.6	30.3		7.1	7:1		5.3	4:1
HY-826-1-6-1	None	4	44.8	.405				18.7	14.9						
	Pythium	4	38.0	.483	.078		38:1	19.4	16.4	.7		21:1	1.5		6:1
A-1-1-2-R-3-2	None	4	62.7	.478				31.1	23.8						
	Pythium	4	41.3	.590	.112		26:1	25.8	23.3		5.3	2069:1		.5	3:
B-1-1-1-1-7-2	None	4	47.3	.523				29.0	23.3						
	Pythium	4	28.0	.500		.023	2:1	14.8	12.7		14.2	348:1		10.6	645:1
G-4-2-1	None	4	71.4	.311				25.6	13.3						
	Pythium	4	38.5	.443	.132		22:1	19.8	10.2		5.8	148:1		3.1	5:1
G-4-4-1	None	4	53.3	.323				20.0	10.0						
	Pythium	4	21.3	.372	.049		221:1	8.9	4.6		11.1	405:1		5.4	2333:1
B-1-1-1-R-8-1-2	None	8	66.0	.510				37.1	25.1						
	Pythium	8	45.0	.560	.050		65:1	25.9	16.8		11.2	1523:1		8.3	59:1
Mean (of the 23 inbred strains)	None		60.0	.462				33.37	23.33						
	Pythium		41.3	.464				22.07	14.88						

The corn was planted on May 16, when the soil temperature was about 56° F. (13.5° C.). The soil was fairly dry. On May 18 there was a 0.56-inch rainfall, followed by a gradual decline in temperature during the following week. The corn germinated, therefore, in a cool, fairly moist soil, comparable to the conditions in the low-temperature tanks in the greenhouse. The reduction in stand and comparative yields are given in Table 5.

Reduction in stand, as compared with the controls, occurred in all cases with one exception. In the control plots a mean stand of 60 per cent was obtained in the 23 inbred strains. The mean stand in the same strains inoculated with *Pythium arrhenomanes* was 41.3 per cent. The reduction in stand due to inoculation was 31 per cent. In the nearly disease-free and Diplodia-infected open-pollinated corn the reductions in stand chargeable to the inoculation were 19.7 and 28 per cent, respectively. When yields were considered, the reduction in stand was more or less counterbalanced by an increased yield per plant in certain of the corn strains. In such cases the Pythium injury evidently took the form of a seedling blight rather than a root rot of the developing plant. In other strains the yield per plant was decreased notwithstanding the reduction in stand. Total yield was generally reduced, the reduction being greater with certain strains of corn than with others. (Figs. 6 and 7.)

The maximum seedling injury following inoculation with *Pythium arrhenomanes* occurred in strain B-1-1-3-R-7-3, where the stand was reduced 60 per cent. Parallel inoculations with *Gibberella saubinetii* caused the maximum reduction in stand of 88.8 per cent in the case of strain K-4-3-1. The mean reduction of sound corn per acre for all strains following inoculation with *P. arrhenomanes* was 36.2 per cent, as compared with 53.3 per cent in the similar Gibberella inoculations.

In the 23 inbred strains the mean yield per plant in the uninoculated controls was 0.462 pound, while that in the series inoculated with *Pythium arrhenomanes* was 0.464 pound per plant. In a parallel series in which *Gibberella saubinetii* was used the mean yield per plant in the uninoculated controls was 0.468 pound and that in the inoculated series 0.478 pound per plant.

Weather Conditions and Experiments in 1925

Weather conditions in the spring of 1925 did not closely parallel those of 1924. The mean air temperature for the entire month of May was 59.2° F., 3.7° below normal. It was the first nine days of the month, however, which were decidedly below the average. The average for the second half of the month was practically normal. Precipitation for the entire month was very low. During the first two weeks of June the mean maximum air temperature rose to 89°. Precipitation again was below normal. The months of May and June, 1925 (figs. 8 and 9), were characterized by a higher mean temperature with wider fluctuations and with less precipitation than in 1924.

In 1925 five inbred lines of corn were planted on two dates. Each line was inoculated at the time of planting with *Pythium arrhenomanes* and *Gibberella saubinetii* in parallel series, accompanied by uninoculated rows which served as controls. The first planting was made on May 12 in a cool, very dry soil. There was a light rain on May

FIG. 6.—Yellow dent corn plants of a strain relatively susceptible to *Pythium arrhenomanes*, grown at Bloomington, Ill., in 1924. A, Inoculated with *P. arrhenomanes* at time of planting; B, uninoculated

FIG. 7.—Yellow dent corn plants of a strain resistant to *Pythium arrhenomanes*, grown at Bloomington, Ill., in 1924. A, Inoculated with *P. arrhenomanes* at time of planting; B, uninoculated

16, sufficient to permit the germination of the seed, but still the soil was dry. The second planting was made on June 4 in a warm, dry soil. This season, then, offered temperature conditions representing low and high soil temperatures, but only low soil moisture in both cases. As a result, the stand was much more irregular, as some

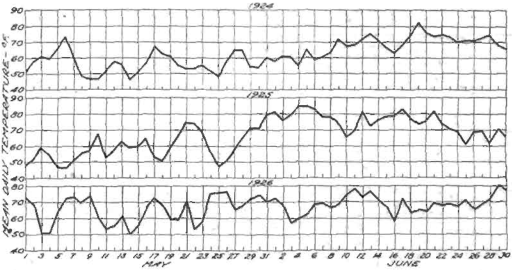


FIG. 8.—Daily mean air temperatures at Bloomington, Ill., during May and June, 1924, 1925, and 1926, respectively

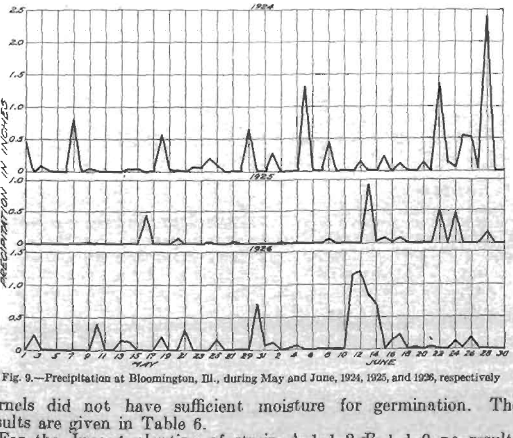


FIG. 9.—Precipitation at Bloomington, Ill., during May and June, 1924, 1925, and 1926, respectively

kernels did not have sufficient moisture for germination. The results are given in Table 6.

For the June 4 planting of strain A-1-1-2-R-1-1-9 no results with Pythium are presented. At that point in the field pocket gophers and field mice disturbed the stand to such an extent that it was deemed unwise to include the data in the table.

TABLE 6.—*Results of inoculating five inbred strains of yellow dent corn with Pythium arrhenomanes and Gibberella saubinetii, planted near Bloomington, Ill., 1925*

Strains	Date of planting	Inoculum used	Number of replications	Field stand (per cent)	Mean yield per plant (pounds)	Mean yield per acre (bushels)	Reduction in acre yield following inoculation: Bushels	Per cent	Odds
A-1-1-2-R-1-1-9	May 12	None	4	37.4	0.319	16.1			
		Pythium	4	16.0	.536	12.9	3.2	19.9	13:1
	do	None	7	64.0	.583	18.7			
		Gibberella	7	39.7	.543	10.9	7.8	41.7	>9999:1
	June 4	None	4	75.3	.329	37.9			
		Gibberella	4	62.0	.394	33.1	4.8	12.7	19:1
A-1-1-2-R-1-2-3	May 12	None	8	30.5	.257	12.6			
		Pythium	8	4.3	.275	1.7	10.9	86.5	>9999:1
	June 4	None	4	67.7	.229	23.1			
		Pythium	4	27.3	.296	12.2	10.9	47.2	434:1
	May 12	None	8	33.2	.258	13.2			
		Gibberella	8	4.7	.358	2.3	10.9	82.6	>9999:1
	June 4	None	4	69.4	.221	22.6			
		Gibberella	4	71.3	.290	29.6	[a]+7.0	[a]+31.0	257:1
A-1-1-4-1-9-5-6	May 12	None	8	21.7	.205	6.9			
		Pythium	8	3.4	.098	.6	6.3	91.3	>9999:1
	June 4	None	4	56.4	.299	25.9			
		Pythium	4	21.3	.243	8.4	17.5	67.6	37:1
	May 12	None	8	22.2	.194	6.8			
		Gibberella	8	1.3	.000	.0	6.8	100.0	>9999:1
	June 4	None	4	62.4	.306	29.1			
		Gibberella	4	68.7	.296	30.8	[a]+1.7	[a]+5.8	3:1
A-1-1-4-1-8-1-5	May 12	None	8	10.0	.269	4.5			
		Pythium	8	2.0	.083	.5	4.0	88.9	>9999:1
	June 4	None	4	55.0	.347	29.0			
		Pythium	4	44.7	.291	20.3	8.7	30.0	35:1
	May 12	None	8	13.5	.212	5.0			
		Gibberella	8	.3	.050	.1	4.9	98.0	>9999:1
	June 4	None	4	58.4	.356	31.9			
		Gibberella	4	57.3	.359	31.3	.6	1.9	1:1
B-1-1-3-R-10-1-12	May 12	None	8	47.7	.553	39.4			
		Pythium	8	12.0	.482	9.2	30.2	76.6	>9999:1
	June 4	None	2	81.4	.411	50.9			
		Pythium	2	60.0	.496	45.3	5.6	11.0	7:1
	May 12	None	8	48.1	.544	38.7			
		Gibberella	8	33.4	.527	26.7	12.0	31.0	41:1
	June 4	None	4	72.3	.458	49.8			
		Gibberella	4	69.4	.458	47.3	2.5	5.0	2:1

[a] + denotes increase.

The stand of the five inbred strains in both plantings was reduced following inoculation with *Pythium arrhenomanes*. The stand of the earlier planting was reduced to a greater extent than that of the later planting. The mean yield per plant varied with the different strains of corn, but in every case the reduction was greater in the early planting.

Following the inoculations with *Pythium arrhenomanes*, reductions in stand were accompanied by increased yields per plant in both plantings of strains A-1-1-2-R-1-2-3, in the June 4 planting of B-1-1-3-R-10-1-12, and in the one planting of A-1-1-2-R-1-1-9. The other inoculations, both early and late, were followed by a reduced stand and a reduced yield per plant. Parallel inoculations with *Gibberella saubinetii* resulted in as great a reduction in stand in the first planting. In the later planted series, however, injury following the inoculations with *G. saubinetii* was slight, if any, whereas following inoculation with *P. arrhenomanes* there was an appreciable reduction in stand and acre yield.

WEATHER CONDITIONS AND EXPERIMENTS IN 1926

The mean temperature during May, 1926, was higher than that of the two preceding seasons, averaging 2.1° F. above normal. The rainfall (2.37 inches) was 1.96 inches below the average for May for the 36 years on record. June, on the other hand, was a relatively cool month, the mean temperature being 68°, 4° below the normal. Rainfall for the month totaled 4.76 inches, which was a fraction of an inch above the average.

The field experiments at Bloomington, Ill., in 1926 included inoculations of inbred strains of corn with *Pythium arrhenomanes*, as in the previous years, and of several crosses and back crosses of inbred strains. (Table 7.)

TABLE 7.—*Results of inoculating inbred strains and crosses of yellow dent corn with Pythium arrhenomanes, planted near Bloomington, Ill., 1926*

[Average of eight replications]

Strain or cross	Field stand in uninoculated plots (per cent)	Percentage increase (+) or decrease (−) in field stand following inoculation	Odds	Plant yield in uninoculated plots (pounds)	Percentage increase (+) or decrease (−) in plant yield following inoculation	Odds	Acre yield from uninoculated plots (bushels)	Percentage increase (+) or decrease (−) in acre yield following inoculation	Odds
Inbred: A-1-1-4-1-8-1-5	65.3	−4.0	3:1	0.321	−8.1	59:1	32.1	−12.8	40:1
Inbred: A-1-1-4-1-9-5-6	62.7	−2.2	2:1	.351	−8.8	66:1	32.8	−9.1	6:1
Cross, F_1: A-1-1-2-R-1-1-9×A-1-1-4-1-9-5-6	78.7	−3.4	2:1	.659	−.6	1:1	79.2	−5.9	6:1
Back cross: A-1-1-2-R-1-1-9×A-1-1-4-1-9-5-6×A-1-1-4-1-9-5-6	70.0	−14.3	103:1	.508	−7.7	11:1	53.8	−11.6	77:1
Back cross: A-1-1-2-R-1-1-9×A-1-1-4-1-8-1-5×A-1-1-4-1-8-1-5	81.3	−6.5	55:1	.543	−4.6	14:1	64.4	−10.4	103:1
Inbred: A-1-1-2-R-1-1-9	73.3	−5.5	8:1	.383	−1.0	1:1	40.2	−10.2	21:1
Cross, F_1: A-1-1-2-R-1-1-9×B-1-1-3-R-10-1-12	73.3	−10.9	52:1	.732	+4.0	28:1	79.8	−7.1	17:1
Cross, F_2: A-1-1-2-R-1-2-3×B-1-1-3-R-10-1-12	74.7	−23.3	932:1	.456	−1.5	1:1	50.8	−22.8	1249:1
Inbred: B-1-1-3-R-10-1-12	74.7	−25.0	382:1	.376	+1.8	......	41.4	−21.5	117:1
Cross, F_1: A-1-1-2-R-1-2-3-7-×B-1-1-3-R-10-1-12	76.0	−8.8	28:1	.760	+5.4	81:1	86.7	−2.3	3:1
Inbred: A-1-1-2-R-1-2-3-7	66.6	−11.9	59:1	.292	+4.5	2:1	27.7	−2.5	2:1
Cross, F_1: G-8-8-1×A-1-1-2-R-1-1-9	80.0	−1.6	2:1	.722	−3.9	9:1	81.5	−5.4	11:1

In no case did the stand or acre yield in the inoculated plots equal that in the uninoculated plots. The greatest reduction in stand, 25 per cent (odds 382:1), occurred in strain B-1-1-3-R-10-1-12. This maximum was closely followed by a 23.3 per cent reduction in stand (odds 932:1) in the F_2 cross of A-1-1-2-R-1-2-3×B-1-1-3-R-10-1-12. The percentage reduction in acre yield nearly equaled the reduction in stand in the two instances. Two inbred strains and two crosses gave an increased yield per plant following a reduction in stand.

Of the inbred strains, B–1–1–3–R–10–1–12 produced the highest yield per acre in the uninoculated plots. It showed, however, the greatest reduction in stand and acre yield following inoculation with *Pythium arrhenomanes*. On the other hand, strain A–1–1–2–R–1–2–3–7, with a poor stand and the lowest yield per acre, was reduced only 2.5 per cent (odds 2:1). The F_1 cross between these two strains resulted in the highest yield per acre in the uninoculated plots and only a 2.3 per cent reduction (odds 3:1) in acre yield following inoculation.

DISCUSSION

It is a matter of common observation that thinning the stand, up to a certain point, tends to increase the yield per plant. Kiesselbach (*9*), experimenting with Hogue Yellow Dent corn over a four-year period (1914–1917), found that 1 plant per hill (3,556 per acre) averaged 40.7 bushels per acre, or 0.64 pound per plant. Two plants per hill (7,112 per acre) yielded 49.4 bushels, or 0.39 pound per plant, while 3 plants per hill (10,668 per acre) yielded 52.9 bushels, or 0.28 pound per plant. In other words, under the conditions of his experiments, a uniform two-thirds reduction in stand resulted in an increase in yield per plant of more than 100 per cent.

Just how much increase in yield per plant may be expected from healthy plants under the conditions of the writers' experiments has not been determined. If, however, the reduction in stand is not excessive and the yield per plant following inoculation is below that of the controls, it seems reasonable to assume, on the basis of the greenhouse experiments, that injury to the root system has caused the reduction in yield per plant of the plants not killed in the seedling stage.

Only a very general comparison of the results of field inoculations for the three years can be made, inasmuch as weather conditions for the three years were so different. The mean air temperature for the 10 days following planting in 1924 was approximately 14° C. For the corresponding period following the May 12 planting in 1925 it was 16° rising to 25° for the 10 days after the late planting on June 4. In 1926 the mean temperature for the corresponding period following planting was 19°. The total rainfall for the months of May and June was 10.75 inches in 1924, 2.9 inches in 1925, and 7.1 inches in 1926. Inoculations made before or near the middle of May resulted in considerable reduction in stand, the percentage varying with different strains of corn. Later plantings caused less reduction in stand. Reduction in yield per plant occurred in varying amounts in both early and late plantings, some strains of corn apparently being more susceptible than others.

From the limited field data obtained from parallel inoculations with *Gibberella saubinetii* and *Pythium arrhenomanes* during two seasons, it appears that the percentage reduction in germination is high in the early plantings and that *G. saubinetii* usually reduces the stand to a greater extent than *P. arrhenomanes*. On the other hand, *P. arrhenomanes* may cause a greater reduction in yield per plant in both early and late plantings. *G. saubinetii* attacks the coleorhiza and mesocotyl of the seedling, retarding the development of an extensive root system. *P. arrhenomanes* seldom attacks the mesocotyl but instead rots the smaller feeding roots.

A comparison of the effect of the two fungi on the yield per plant can be made in the few cases in which reduction in stand was practically the same following inoculation with *G. saubinetii* and *P. arrhenomanes*, as shown in Table 8.

TABLE 8.—*Results of inoculating three inbred strains of corn with Gibberella saubinetii and Pythium arrhenomanes, planted May 16, 1924*

Strain	Inoculum used	Field stand		Mean yield per plant (pounds)	Odds [a]
		Number of plants	Mean (per cent)		
B-1-1-3-R-7-3	None	50	60.0	0.563	
	G. saubinetii	18	24.0	.736	>9999:1
	P. arrhenomanes	18	24.0	.514	1666:1
G-8-8-1	None	55	72.0	.502	
	G. saubinetii	42	56.0	.429	5309:1
	P. arrhenomanes	41	54.7	.384	>9999:1
A-1-1-2-R-3-2	None	46	60.9	.513	
	G. saubinetii	32	42.7	.595	3:1
	P. arrhenomanes	31	41.3	.590	26:1

[a] For difference in yield between uninoculated and inoculated plants.

Although both *Pythium arrhenomanes* and *Gibberella saubinetii* (2) cause the greatest seedling injury at low temperatures, resistance or susceptibility to these two organisms does not seem to be combined necessarily to the same degree in a given strain of corn.

SUMMARY

Pythium injury to corn may be manifest as (1) a rot of the embryo, preventing germination; (2) as a seedling blight after emergence; or (3) as a root rot that tends to reduce the size, vigor, and yield of the maturing plant.

The causal organism has just been described by Drechsler as *Pythium arrhenomanes* n. sp.

Unlike *Diplodia zeae* and *Gibberella saubinetii*, *Pythium arrhenomanes* attacks the mesocotyl of the seedling only in the late stages of the disease, and then only in cases of severe attack. Infection takes place at the tip of the rootlet. It produces a soft rot that involves first the cortex and later the vascular elements.

Growth of the fungus on potato-dextrose agar takes place between 4° and 40° C. The optimum for vegetative growth lies between 30° and 36°.

Experiments in which open-pollinated and inbred corn was inoculated with *Pythium arrhenomanes*, at the time of planting, in soil-temperature control tanks indicate that soil temperatures near 16° C. or lower, together with high soil moistures, are so favorable for infection that germination of corn kernels may be prevented or seedling blight produced. When inoculated corn is not killed in the seedling stage, the height and dry weight of tops of the plants are reduced. The highest percentage reduction occurred at the lower temperatures. Evidences of differing degrees of resistance or susceptibility were manifest in different inbred strains of corn.

Field experiments during three seasons tend to confirm the results obtained in the greenhouse.

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VARIATION IN SEED FUZZINESS ON INDIVIDUAL PLANTS OF PIMA COTTON[1]

By THOMAS H. KEARNEY, *Principal Physiologist in Charge*, and GEORGE J. HARRISON, *Chief Scientific Aid, Office of Alkali and Drought Resistant Crops, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

It was shown in an earlier paper [2] that plants of the Pima variety of American Egyptian cotton, grown under irrigation in Arizona, produced longer lint in the bolls borne on the upper part of the plant than in the lower bolls. The data from which this conclusion was drawn were obtained as follows: The fruiting branches on each of 10 plants were numbered consecutively from the lowest to the highest, the boll borne at the second node of each alternate fruiting branch was harvested when mature, and the lint from each boll was measured. The 10 plants were taken as one array, since they did not differ significantly in their mean lint lengths. The coefficient of correlation (not given in the paper cited) between number (height) of the fruiting branch and lint length for this population was 0.491 ± 0.047, indicating a rather marked tendency for the lint to increase in length from the base to the summit of the plant.[3]

Another character showing considerable variation on the individual cotton plant is the fuzziness of the seeds. A strong tendency to greater fuzziness of the seeds produced in the lower bolls has frequently been noted in Pima cotton grown in Arizona. In an endeavor to obtain statistical evidence of the magnitude and significance of this difference the procedure followed was similar to that used in studying the variation in length of lint.

MATERIAL AND METHODS

Ten plants of an inbred family of the Pima variety (family 5-11) were taken at random at the United States Field Station, Sacaton, Ariz., in 1927. The only conscious selection was the rejection of plants on which very heavy shedding had taken place. On December 10, about 10 days after the first killing frost, every open boll on each of these plants was picked separately, and its position on the plant was recorded on a label attached to the sample. The position was fixed by numbering the fruiting branches consecutively from base to

[1] Received for publication July 13, 1928; issued November, 1928.

[2] KEARNEY, T. H., and HARRISON, G. J. LENGTH OF COTTON FIBER FROM BOLLS AT DIFFERENT HEIGHTS ON THE PLANT. Jour. Agr. Research 28: 563-565, illus. 1924.

[3] *It was noted, however, that the bolls borne on the very highest fruiting branches, which opened late in* the season, contained somewhat shorter lint than the bolls borne on most of the upper fruiting branches. This is clearly brought out in the following: KEARNEY, T. H., and HARRISON, G. J. Op. cit., p. 564, fig. 1, Table 2. In this connection, E. C. Ewing of the Delta and Pine Land Company of Mississippi has informed the writers that in the Delta region of Mississippi the lint of long-staple upland varieties of cotton is shorter in the later than in the earlier pickings. The difference in behavior may be due to the fact that in Arizona cotton is grown under irrigation and is not normally subject to drought late in the season, which Mr. Ewing states is of frequent occurrence in Mississippi.

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apex of the plant and by numbering the nodes of the fruiting branches consecutively from the base of the branch outward. The seed cotton from each boll was ginned separately. After ginning, the packets containing the seeds from each boll were placed on a table with the labels face downward, so that the grading in respect to fuzziness could be done without prejudice.

The grading was done by comparing the seeds with a series of standard samples representing the range of grades (1 to 9) that has been observed in the Pima variety. When there was any doubt as to the grade to which a sample belonged, it was always assigned to the higher grade. The grades represented in this material were 3 to 9, and these extremes are shown in Figure 1, which is reproduced from a photograph of the standards themselves.

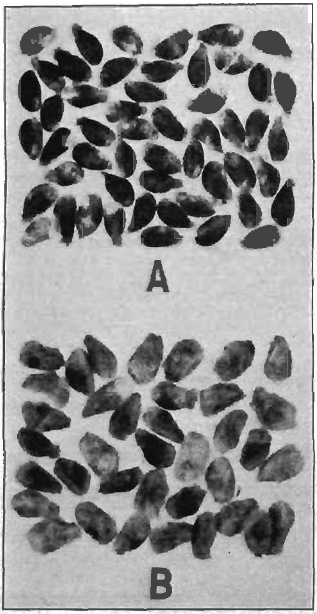

FIG. 1.—Seed fuzziness standards for Pima cotton, showing grades 3 (A) and 9 (B), these being extremes represented on the plants that afforded the data considered in this paper

On January 5, 1928, the bolls were collected from 10 plants of another but closely related Pima family (family 5–12), and this additional material was treated similarly in all respects.

The considerable individual variation in seed fuzziness which characterizes this variety of cotton, manifesting itself even on the plants of inbred and otherwise uniform families, is shown by the fact that on 13 of the 20 plants all seven grades (3 to 9) were represented, while the remaining plants each showed a range of six grades.

STATISTICAL CONSTANTS FOR THE TWO FAMILIES

The mean and standard deviation for each plant and for all 10 plants of each family as one array are given in Table 1. In neither group was the population perfectly homogeneous. The means of two individuals of family 5–11 differed significantly from the mean of the population, the mean of plant 8 having been lower and that of plant 10 higher. In both cases the departure was about five times its probable error. In family 5–12, plant 11 had a significantly higher mean and plants 18 and 19 had significantly lower means than the

mean of the population, the departure having been respectively 6.3, 5.3, and 5.3 times its probable error. Nevertheless, for the reason to be given presently, it was decided to treat the 10 plants of each family as one array in determining the correlations.

Comparison of the means of the two populations, as given at the bottom of Table 1, shows that family 5–12 had slightly fuzzier seeds than family 5–11, and that the difference in mean grade was significant, having amounted to 8.6 times its probable error.

TABLE 1.—*Statistical constants for grade of seed fuzziness of 20 plants of Pima cotton*

Family and plant	Number of bolls	Mean grade	Standard deviation [a]	Family and plant	Number of bolls	Mean grade	Standard deviation [a]
Family 5-11:				Family 5-12:			
No. 1	41	6.0±0.19	1.80	No. 11	76	7.1±0.10	1.34
No. 2	51	5.7± .14	1.47	No. 12	69	6.5± .14	1.71
No. 3	52	5.8± .14	1.54	No. 13	67	6.7± .13	1.57
No. 4	62	5.8± .12	1.37	No. 14	68	6.0± .14	1.78
No. 5	54	6.4± .17	1.89	No. 15	72	6.5± .12	1.48
•No. 6	47	5.8± .15	1.49	No. 16	57	6.6± .14	1.59
No. 7	46	5.9± .19	1.88	No. 17	67	6.6± .14	1.74
No. 8	37	4.8± .20	1.83	No. 18	63	5.6± .15	1.82
No. 9	46	5.6± .16	1.61	No. 19	60	5.6±9.15	1.70
No. 10	51	6.6± .14	1.54	No. 20	80	6.7± .13	1.70
As one array	487	5.87± .05	1.67	As one array	679	6.42±.04	1.70

[a] The standard deviations and probable errors of the individual plant means have been increased by using Pearson's correction for the standard deviation when the numbers are small. See PEARSON, K., ON THE DISTRIBUTION OF THE STANDARD DEVIATIONS OF SMALL SAMPLES: APPENDIX I TO PAPERS BY "STUDENT" AND R. A. FISHER. Biometrika 10: 529. 1915.

CORRELATION BETWEEN SEED FUZZINESS AND POSITION OF THE FRUITING BRANCH

For the 10 plants of each family as one array, the correlations were determined between the height of the fruiting branch as indicated by its number from the base and the grade of fuzziness of the seeds from the bolls borne on the branch in question. The correlations with the height of the branch were determined for (A) the average of the grades of all bolls on the branch and (B) the grade of the boll at the first node of the branch. Since in many cases node 1 was vacant, because of shedding, the numbers are smaller for correlations B than for correlations A. The coefficients obtained are given in Table 2. They are in all cases negative and highly significant, indicating a strong tendency for the fuzziness of the seeds to diminish from base to apex of the plant.

TABLE 2.—*Coefficients of correlation of the grade of seed fuzziness with the position (number from the base of the plant) of the fruiting branch on 20 plants of Pima cotton in 1927*

Height of branch correlated with seed fuzziness as expressed by—	Family 5-11 (plants 1 to 10)			Family 5-12 (plants 11 to 20)		
	n	r	r/E	n	r	r/E
Average for all bolls on the branch (A)	194	−0.652±0.028	23	216	−0.638±0.027	23.5
Boll at node 1 only (B)	186	− .557± .034	16	164	− .711± .037	19

The observed and fitted regressions of seed fuzziness on the height of the fruiting branch in the two Pima families are shown in Figure 2. The fitted regressions indicate an average decrease in seed fuzziness for each successive pair of branches up the plant amounting to 0.28 grade in family 5–11 and 0.22 grade in family 5–12.

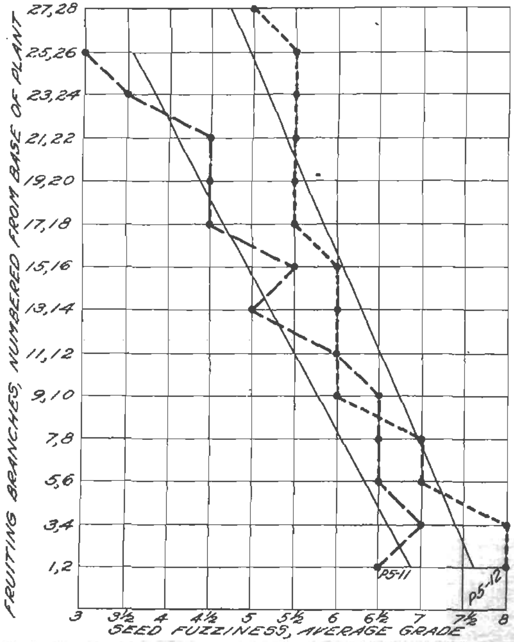

FIG. 2.—Observed and fitted regressions of seed fuzziness on the height of the fruiting branch in Pima cotton. The fitted regressions indicate the average decrease in fuzziness on successively higher fruiting branches. The broken line represents the observed regression for the 10 plants of family 5-11 and the dotted line the observed regression for the 10 plants of family 5-12. The solid lines represent the fitted regressions

It was mentioned in a preceding paragraph that certain plants in each population were somewhat aberrant in their means for seed fuzziness. The correlations between the height of the fruiting branch and seed fuzziness (average of the grades of all bolls on the branch) were determined separately for these individuals and are shown in comparison with the corresponding coefficients of the respective

populations (10 plants as one array) in Table 3. The individual coefficients in no case differ significantly from the coefficient of the whole population and, like the latter, are in every case negative and significant. Therefore, it seemed justifiable to include these plants in the populations on which were determined the correlations given in Table 2.

TABLE 3.—*Coefficients of correlation between the height (number) of the fruiting branch and seed fuzziness for the apparently aberrant plants in Pima families 5–11 and 5–12 and for the 10 plants of each family as one array*

Family and plant	Coefficient	Family and plant	Coefficient
Family 5–11:		Family 5–12:	
Plant 8	−0.699±0.084	Plant 11	−0.597±0.091
Plant 10	− .520± .110	Plant 18	− .629± .093
		Plant 19	− .791± .055
Ten plants as one array	− .652± .028	Ten plants as one array	− .638± .027

CORRELATION BETWEEN SEED FUZZINESS AND POSITION OF BOLL ON FRUITING BRANCH

In order to ascertain whether there is a tendency for the seed fuzziness to vary consistently at different points on the same fruiting branch, the following procedure was adopted: The nodes of the individual fruiting branches were numbered consecutively outward, and the correlation between the node number of the boll and the grade of fuzziness of the seeds contained in it was determined without regard to the position of the branches themselves. The correlation for 487 fruiting-branch nodes in family 5–11 (plants 1 to 10) gave a coefficient of −0.290±0.028 (r/E 10.3); and the correlation for 679 fruiting-branch nodes in family 5–12 (plants 11 to 20) gave a coefficient of −0.420±0.021 (r/E 20.0). There is, therefore, a tendency for the bolls at nodes near the base of the branch to contain fuzzier seeds than the bolls farther out on the branch.

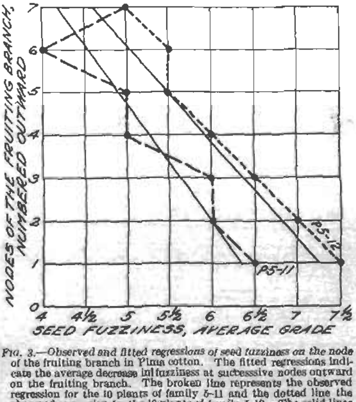

FIG. 3.—Observed and fitted regressions of seed fuzziness on the node of the fruiting branch in Pima cotton. The fitted regressions indicate the average decrease in fuzziness at successsive nodes outward on the fruiting branch. The broken line represents the observed regression for the 10 plants of family 5–11 and the dotted line the observed regression for the 10 plants of family 5–12. The solid lines represent the fitted regressions

The observed and fitted regressions of seed fuzziness on the number of the node of the fruiting branch in the two Pima families are shown in Figure 3. The fitted regressions indicate an average decrease in seed fuzziness for each successive node outward on the branch amounting to 0.36 grade in family 5–11 and 0.43 grade in family 5–12.

Additional and conclusive evidence of this relation was obtained by comparing, for all branches retaining three or more bolls, the seed fuzziness grade of the innermost and the outermost boll. The numbers of such branches available were 78 on the 10 plants of family 5–11 and 113 on the 10 plants of family 5–12. The means of the grades of the innermost and outermost bolls are stated in Table 4, the probable errors of the differences having been computed from the array of differences between pairs (innermost and outermost boll of each branch). It is evident that the inner bolls averaged about two grades fuzzier than the outer bolls and that the differences are highly significant.

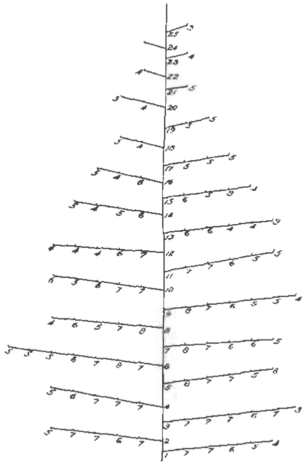

Fig. 4.—Composite diagram of 10 plants of Pima cotton (family 5-11), showing the average grade of seed fuzziness of the boll at each node of each fruiting branch. The vertical line represents the main stalk, and the numbers along it are those of the successive fruiting branches. The lines to the right and the left represent the fruiting branches, and the figures along them show the average of the grades of seed fuzziness (nearest whole number) for all bolls in the population which were borne at the position indicated

The relation between the position of the boll and the fuzziness of the seeds is shown in Figure 4, which is a composite diagram of the 10 plants of family 5–11. The vertical line represents the main stalk and the figures along it are the numbers of the fruiting branches. The lines to the right and the left represent the branches, and the figures along them show the average of the grades of seed fuzziness (nearest whole number) of all bolls in the population borne at the position indicated. There having been considerable shedding of buds and bolls on all of these plants, the averages in several cases are based on only one or two bolls. This doubtless accounts for most of the irregularity of the gradation in seed fuzziness upward on the plant and outward on the branches.

TABLE 4.—*Mean grade of fuzziness of seeds from bolls nearest and farthest from the base of each fruiting branch bearing three or more bolls*

Position of bolls	Mean grade of seed fuzziness in—	
	Family 5-11 (plants 1 to 10)	Family 5-12 (plants 11 to 20)
Nearest base of branch	7.21	7.66
Farthest out on branch	5.04	5.76
Difference	2.17±0.15	1.90±0.12
D/E	14.5	16

SUMMARY AND CONCLUSIONS

Evidence is presented in this paper which shows that in Pima cotton grown in Arizona there is pronounced variation, as between bolls borne in different positions on an individual plant, in the quantity of fuzz or short hairs on the seeds. Rather high and very significant negative correlations between the height of the fruiting branch and the grade of fuzziness of the seeds borne thereon indicate a strong tendency for the bolls on the lower fruiting branches to have fuzzier seeds than the bolls on the higher branches. Lower but still rather significant negative correlations between the number of the node on the individual fruiting branch and the fuzziness of the seeds contained in the boll at that node show that seeds produced nearest the base of the branch tend to be fuzzier than those produced farther out on the branch.

These positional relations suggest that better conditions of nutrition may be conducive to greater development of fuzz on the seeds, since it is the bolls borne on the lower part of the plant and near the base of the fruiting branch that usually have the fuzziest seeds. Conversely, the quantity of fuzz tends to be smallest on seeds produced farthest from the roots and from the main stalk of the plant. It is not improbable, however, that changes in temperature or in the length of day as the season advances may be important factors, since the bolls produced near the base of the plant and of the fruiting branch develop while the day and night temperatures are highest and the days are longest; whereas temperatures, particularly night temperatures, are appreciably lower and the days are appreciably shorter during the period of development of the bolls near the top of the plant and farthest out on the fruiting branches.

The flowers that open late in the season, hence on the highest fruiting branches and at the farthest nodes of lower branches, are smaller and paler colored than the earlier flowers. So far as the writers know, no comparison has yet been made of the size and weight of the bolls and weight of the seeds in relation to position on the plant. Data are at hand, however, in regard to the time required for maturation of the boll. Martin, Ballard, and Simpson [4] found that in several types of cotton the maturation period (number of days from opening of the flower to opening of the boll) increases as the season advances. It is stated that in Pima cotton grown at Phoenix, Ariz., C. J. King

[4] MARTIN, R. D., BALLARD, W. W., and SIMPSON, D. M. GROWTH OF FRUITING PARTS IN COTTON PLANTS. Jour. Agr. Research 25: 203-206. 1923.

observed the maturation period to increase from 54 days for flowers opening in July to 82 days for flowers opening in September. Observations on Pima cotton by R. H. Peebles and Max Willett at the United States Field Station, Sacaton, Ariz., in 1921, showed the maturation period to lengthen from an average of 54.5 days for 618 flowers opening in July to an average of 74.5 days for 981 flowers opening in September.

Most of the flowers opening in July are borne on the lower fruiting branches and on the basal nodes of the branches, whereas most of the flowers opening in September are borne on fruiting branches near the top of the plant or far out on lower branches. Consequently, as in the case of seed fuzziness, it would be possible only by experimental control of temperature and of the daily period of illumination to ascertain the relative importance of these factors and of conditions with respect to nutrition in determining the length of the maturation period.

If relatively unfavorable nutritional or meteorological conditions are responsible for the slower maturation of the bolls from flowers produced late in the season and high on the plant and for the smaller quantity of fuzz on the seeds contained in these bolls, it is difficult to account for the fact, mentioned in the introduction to this paper, that on plants of Pima cotton grown in Arizona the lint contained in the upper bolls was found to be longer than that in the lower bolls. A negative correlation between lint length and seed fuzziness would be expected if the increase in length of lint upward on the plant is a general phenomenon, but in the two instances in which the correlation between this pair of characters has been determined for Pima cotton in Arizona the coefficients obtained were positive, although not significant (r 0.046 ± 0.051 and r 0.073 ± 0.047). It would be unprofitable to speculate further concerning this apparent discrepancy until determinations of lint length and seed fuzziness have been made on the same individual bolls borne in different positions on the plant.

The magnitude of the variation in seed fuzziness from boll to boll on the same individual is noteworthy. Of the 20 plants examined in this investigation, 13 showed a range of seven grades and 7 showed a range of six grades, the total variation which has been observed in the Pima variety being represented by nine grades. It is evident that in comparing different strains or varieties of cotton in respect to this character, and in genetic studies, particular care must be used in collecting the seeds. These should be taken either from bolls occupying corresponding positions on the several individuals or from a sufficient number of bolls on all parts of the plant to afford an average sample.

ZONATE EYESPOT OF GRASSES CAUSED BY HELMINTHOSPORIUM GIGANTEUM[1]

By Charles Drechsler

Associate Pathologist, Office of Vegetable and Forage Diseases, formerly with *Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Helminthosporium giganteum Heald and Wolf (*6*)[2] was described in 1911 from Texas, where it was found occurring on diseased Bermuda grass (*Cynodon dactylon* L.), as the cause of lesions evidently of the eyespot type. The writer included a discussion of the fungus in a comparative account published in 1923 (*4*), in which its occurrence on goose grass (*Eleusine indica* (L.) Gaertn.) and quack grass (*Agropyron repens* (L.) Beauv.) was noted and the peculiar mode of germination characteristic of its conidia was described. In an abstract that appeared somewhat earlier (*3*) the parasite had been reported on nearly a score of additional species of grasses and an explanation offered of its method of extension as prevailing in the development of a much more destructive type of injury observed on several hosts and designated as zonate eyespot. In the present paper the degree of injury sustained by the grasses on which the fungus has hitherto been observed to occur naturally will be more fully discussed, together with certain features pertaining to the morphology and develoment of the parasite.

DISTRIBUTION AND SEASONAL OCCURRENCE OF PARASITE

Such fragmentary information concerning the distribution of *Helminthosporium giganteum* as it has been possible to obtain in occasional field trips undertaken for other purposes indicates that in the United States it is largely restricted to the southern and middle latitudes. Collections made by the writer at Seaford, Del., in August, 1922; at Hurlock, Md., in August, 1923; in the District of Columbia and neighboring sections of Virginia and Maryland in 1922, 1923, 1924, 1925, and 1926; and at Menfro, Mo., in August, 1924, provide clear evidence that the parasite is not limited to a strictly southern distribution. Precisely how much farther north its natural distribution extends is not known except that efforts to find the fungus in the western portion of Long Island during the seasons of 1920 and 1921, in the vicinity of Vincennes, Ind., in August, 1924, and in the vicinity of Allentown, Pa., in September, 1925, were unsuccessful, although grasses capable of serving as congenial hosts

[1] Received for publication Apr. 27, 1927; issued November, 1928.
[2] Reference is made by number (italic) to "Literature cited," p. 492.

were present in quantity.[3] On the other hand, except apparently for mountainous and elevated regions, the parasite has been found of widespread occurrence in the Southeastern States from Virginia to Florida, while its prevalence in a number of localities in southeastern Missouri, visited in August, 1923, suggests a parallel distribution in the Mississippi Valley.

Helminthosporium giganteum shows much variability in the abundance of its occurrence from season to season. During the summer of 1922 it appeared in the vicinity of Washington, D. C., as probably the most destructive single fungous parasite affecting the Gramineae as a family, its attack on several of its hosts being of extreme severity, while on some others its attack, though less destructive, was nevertheless severe. During the seasons of 1923, 1924, 1925, and 1926 the fungus was far less prevalent and, in general, of only minor importance, although in a number of situations, as along the banks of the Chesapeake & Ohio Canal, it reappeared from year to year in nearly undiminished quantity. In addition to irregularity with respect to seasonal occurrence, it exhibits much less uniformity in local distribution than the large majority of parasitic fungi. Even in the season of 1922, when in many locations stands of Bermuda grass were all but killed as a result of the ravages of the parasite, it was not an unusual experience to find other stands of the same grass within a distance of less than 100 meters free of injury. Such pronounced inequality of distribution has been found characteristic of the fungus wherever adequate observations have been made, prevailing apparently in southeastern Missouri exactly as in Virginia and Maryland.

For such localized distribution a partial explanation may be offered. Compared to some of the more nearly ubiquitous types of foliar parasites—as, for example, *Helminthosporium sativum* P. K. & B.—*H. giganteum* produces even under favorable conditions a relatively small number of spores. These spores, as has been pointed out previously (*4, p. 676*), are the shortest lived spores of any species of Helminthosporium hitherto encountered by the writer. There is evidence, too, that they are not well adapted for extensive dispersal. The dissemination of conidia of *H. giganteum* can be studied to advantage in situations where a single isolated infected stand of a favorable host on which active sporulation is taking place is found adjacent to, or surrounded by, species of grasses allowing the production of only incipient lesions devoid of fructifications. The abundance or scarcity of sterile lesions on the uncongenial grasses in such circumstances may be regarded as a reliable index of the quantity of spores reaching any particular spot from the stand of the congenial species of grass. The numbers of such lesions fall off rapidly beyond distances of 1 to 2 meters; few are to be observed at a distance of 5 meters; only a vanishing quantity can be found at 10 meters, while none have ever been observed at a distance of 20

[3] As a pest affecting creeping bent grass in putting greens of golf courses, the fungus was found to occur during the season of 1928 in widely separated localities in the northern tier of Middle Western States. In the three localities where the writer had occasion to make observations, viz. La Fayette, Ind., Detroit, Mich., and Wooster, Ohio, natural stands of susceptible hosts (as, for example, quack grass) showed no evidence of infection with zonate eyespot. Nor were any signs of attack by *Helminthosporium giganteum* evident in the creeping bent immediately surrounding affected putting greens but not exposed to artificial watering. It appears highly probable that the success of the fungus well north of what would seem to be its natural range is contingent on the copious irrigation usual in the management of greens. As the grass is generally propagated by stolons, and since these to a large extent have been distributed from sources within the natural range of the parasite, the means by which the introduction of the latter into northern localities has been effected are sufficiently obvious.

meters. It is scarcely to be doubted that the unusual size and consequent relatively great weight of the conidia are in large part responsible for the restriction in spread. Obviously these bodies would scarcely remain suspended long in the somewhat quiet atmosphere often associated with the light, sustained precipitation that provides optimum conditions for their germination.

OVERWINTERING OF PARASITE

In the vicinity of the District of Columbia *Helminthosporium giganteum* ceases to develop vegetatively or to produce conidia with the advent of cool weather during the early part of October. The fungus appears to overwinter as dormant mycelium. At intervals during the spring of 1923, quack-grass leaves of the previous season, with well-developed lesions, were collected near Cabin John, Md., brought into the laboratory, and incubated in a moist chamber. Fresh conidia were obtained in this way until early in May, when, with the appearance of lesions on the new quack-grass foliage, the trials were discontinued. Although it was never possible to determine definitely that fresh conidia were not proliferated from the old conidiophores, most of the conidia were apparently produced on new conidiophores, and perhaps all may have had such origin.

DEVELOPMENT OF LESIONS IN RELATION TO SPECIFIC SUSCEPTIBILITY

Collectively the various grasses (pls. 1–7) found to show evidence of attack by *Helminthosporium giganteum* under natural conditions manifest the widest range in degree of susceptibility. The most general manifestation of an individual infection is the appearance on the foliage of a minute longitudinally elongated spot, the size and coloration of which vary with the host. In *Muhlenbergia schreberi* Gmel., for example, this spot first becomes visible as a sharply defined very dark speck, often not exceeding 0.05 mm. in width and 0.2 mm. in length. (Pl. 5, N–R.) Through subsequent enlargement it may attain a length of approximately 1.2 mm. and a width of 0.1 to 0.2 mm., then often revealing within these relatively minute dimensions a decolorized central region. In other hosts, where discoloration is less intense, the spots, when first recognizable, may be somewhat larger and less sharply delimited than in *M. schreberi*, and the primary lesions, before attaining definitive size, may become several times larger. The fading of the central region to yield the eyespot type of lesion generally distinctive of the disease occurs with less regularity in some hosts than in others. *Panicum dichotomoflorum* Michx., for example, as a result of frequent omission of this development, often exhibits the spot-blotch type in larger number. In timothy (*Phleum pratense* L.) the lesion is practically devoid of dark discoloration, being present as a dead region from which the normal green coloration has disappeared.

The mere occurrence of the eyespot type of lesion on a grass does not necessarily provide evidence of its suitability to serve as a host to *Helminthosporium giganteum*. In the case of many species of grasses such lesions have never been discovered except in mixed stands with more congenial hosts, on which the parasite is not only present but sporulating abundantly as well. In Virginia, Maryland, and Mis-

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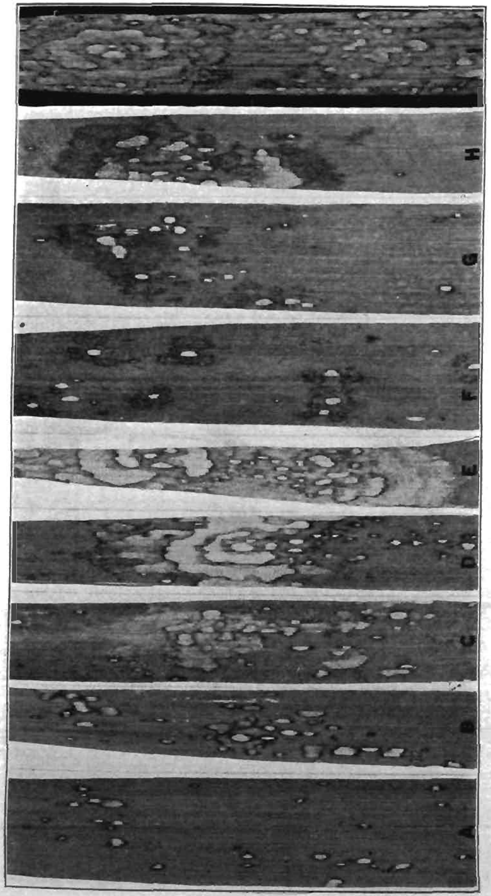
souri, Bermuda grass, goose grass, and quack grass appear to serve most frequently as sources of infection, most of the writer's observations on the response of other grasses having consequently been carried out when the latter occurred in immediate proximity to these widely distributed weeds, and most frequently as intimate admixtures. Under such circumstances the foliage of uncongenial hosts may bear an abundance of spots, as occurs, for example, in *Panicum clandestinum* L., or a few more remotely scattered ones may be produced, as in timothy. In any case the tissue involved never appears to give rise to fructifications of the fungus, nor can such structures ordinarily be obtained by incubation in a moist chamber. If the affected leaves are still green, the identification of the parasite may usually be accomplished, though somewhat laboriously, by isolating it from bits of tissue excised from the margins of lesions and planted on a suitable culture medium after surface sterilization followed by thorough washing with sterile water.

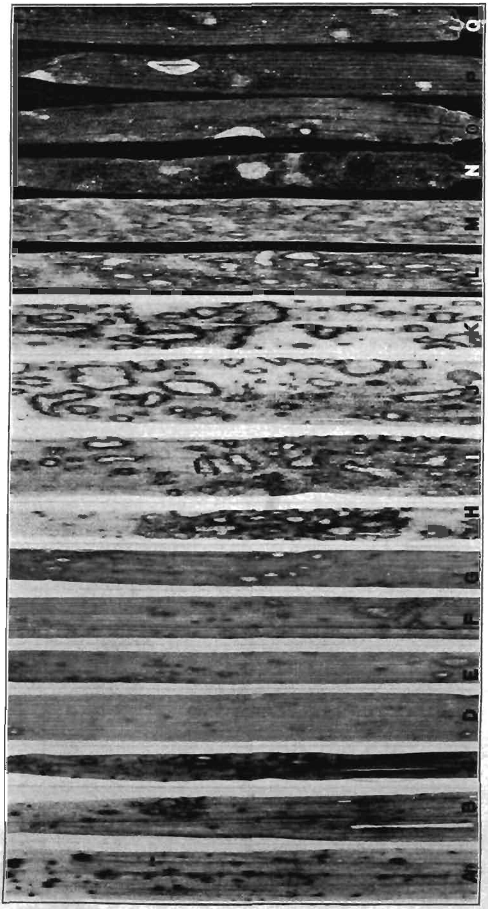
A readier and more certain method of determining the parasite under consideration as the effective causal agent is that of direct microscopic examination; for, owing to the extraordinary size of the conidia, the evacuated collapsed membranes of the individual spore usually can be discerned without the least difficulty on one surface or the other of the lesion that it produced. The examinations, it may be mentioned, revealed that very generally the greater number of infections result from spores on the upper or adaxial surface of the leaf, a fact to be attributed, perhaps, to the somewhat more effective exposure of this surface to air-borne bodies. On whatever surface the spore membranes may be found, however, they are always securely fastened to the host by the evacuated germ tubes, so that alcohol and clearing agents may be employed to improve the optical features of the material without incurring any risk of washing off the structures in question.

Most frequently the evacuated spore membranes occupy an approximately median position relative to the foliar lesion, the germ tubes from both ends having evidently been equally active in killing regions of tissue that became sufficiently extensive to coalesce into one. However, in the case of hosts in which, as in *Muhlenbergia schreberi*, the lesions are clearly delimited and very narrow, such coalescence does not always take place, particularly when the conidium is oriented in a direction transverse to that of the leaf. In such cases two distinct lesions separated by a distance not usually exceeding 0.3 mm., yet clearly evident as two to the naked eye, result from the germination of the single conidium. This condition is infrequent in fungous diseases generally, and manifestly is not readily possible with fungi having spores of more ordinary dimensions. A similar pairing is exhibited also by newly developed lesions on more favorable hosts (pl. 1, A), but owing to the enlargement and fusion of the individual spots the binary arrangement later becomes obliterated. Sometimes a spore may be more or less eccentric in position with reference to the lesion produced by it, evidently as a result of irregularities in germination due to accidents attending the process or to the previous death of some of the segments.

Among the uncongenial hosts considerable difference exists with respect to the number of lesions produced under circumstances equally favorable for infection. *Panicum dichotomoflorum* and *Muhlenbergia*



Portions of leaves of *Phalaris arundinacea* attacked by *Helminthosporium giganteum*. A–E, Series of specimens showing infection of increasing severity; F–H, Water-soaked zones surrounding lesions as a result of incubation in moist chamber for 16 hours; I, Leaf completely involved in extensive lesions with conspicuous zonate markings; × 2



Leaves of various grasses attacked by *Helminthosporium giganteum*. A–C, *Agropyron elongatum*, × 2; D–H, *A. intermedium*, × 2; I–M, *A. repens*, × 2; N–Q, *Agrostis stolonifera*, × 4

schreberi, when found in mixed stands with heavily infected Bermuda grass, are generally very liberally peppered with discolored spots, the lesions here being often as abundant as on the congenial hosts. On the other hand, *Panicum gattingeri* Nash, as well as timothy and Kentucky bluegrass (*Poa pratensis* L.), under the same conditions exhibit only a meager sprinkling of spots. Neither crabgrass (*Digitaria sanguinalis* (L.) Scop.) nor *Chaetochloa lutescens* (Weigel) Stuntz were ever found spotted by the fungus in the vicinity of Washington, D. C., during the five successive seasons in which observations were made. Near Kennett, Mo., however, lesions attributable to conidia of the parasite were found on both, though, to be sure, in small number. It is probable that such facts of presence or absence on a particular host may involve only casual details of distribution. On the other hand, they may point toward differences in environmental conditions, or toward possible differences in the biological constitution either of the parasite or of the grass host. In the absence of more precise information, it may be mentioned in this connection that, on the whole, the distribution of the fungus in nature does not suggest the existence of physiological varieties or races paralleling generic divisions in the Gramineae.

In the case of the more congenial hosts, the early stage in the establishment of the parasite is closely similar to the development of the small lesions just described. (Pl. 1, A, B.) The hyphae proceeding from the germinating conidium here also bring about the discoloration and death of a limited tract of tissue. On isolated eyespot lesions of such origin, fructifications of the fungus do not ordinarily arise. However, when, as in Bermuda grass, these spots become numerous and crowded, causing the leaf involved to wither somewhat generally, conidiophores appear in considerable abundance, from intervening regions as well as from the bleached areas included within the lesions.

A generally more copious production of sporophores and spores takes place on leaf tissue directly killed by the parasite as a result of a peculiar type of secondary development. This type of development is most strikingly exemplified on *Phalaris arundinacea* L. and appears to be dependent on the presence of liquid water on the surface of the leaf. When, because of heavy dews or prolonged drizzling rains, a layer of water persists 12 hours or more on infected foliage of reed canary grass, many of the eyespot lesions will be found surrounded by an enlarging water-soaked zone. (Pl. 1, F, G, H.) Microscopic examination of the surface of the leaf reveals the presence of hyphae arising near the edge of the original lesion and traversing the water-soaked zone radially to its margin, giving off branches in their course. These superficial filaments adhere very closely to the epidermis of the host, and would seem to communicate with the interior of the leaf by branches penetrating the epidermis, although the direct optical evidence for such communication is far from satisfactory. In any case, the water-soaked zone is rather accurately coextensive with the region included in the centrifugal growth of superficial hyphae. With the disappearance of the layer or film of water on the advent of drier conditions, growth of the superficial mycelium ceases and the zone of water-soaked tissue dries up, thus becoming the peripheral belt of the enlarged lesion. (Pl. 1, D.) When, as in the season of 1922, weather conditions are such as to permit repeated occurrence of the same cycle of development, many of the leaves

become entirely involved, the dead foliage, entirely covered with intricately zonate patterns, presenting a most distinctive aspect. (Pl. 1, E, I.) Irregularities in such patterns (pl. 1, D) are attributable, as might be expected, to the casual distribution of the moisture deposited, regions failing to become covered being recorded as interruptions in the zone developed during any particular moist period under consideration. The older leaves of reed canary grass usually show most extensive infection on the blade midway between base and tip. Field inspection has shown that this median portion also becomes more liberally bathed in dew, a fact due apparently to the drooping habit of the distal part and the accumulation of moisture near the keystone position on the resultant arch.

The importance of such secondary development in the biology of the parasite is considerable, as by far the larger portion of the conidiophores and conidia are produced in the extensive regions of host tissue killed thereby. The degree to which secondary development takes place on any host becomes thus a truer measure of its congeniality than the number of infections. Hosts that may well be regarded as congenial include, in addition to Bermuda grass and reed canary grass, *Agropyron repens*, *A. intermedium* Beauv., *A. elongatum* Host, *Bromus inermis* Leyss., *Eleusine indica*, *Echinochloa crusgalli* (L.) Beauv., *Elymus virginicus* L., *Lasiagrostis splendens* Kunth,[4] and *Leersia virginica* Willd. Under favorable conditions all of the grasses mentioned would seem to permit the parasite to propagate itself indefinitely. Sporulation was observed also on leaves of *Eragrostis major* Host and of *Muhlenbergia mexicana* (L.) Trin., though in such meager quantity as to render doubtful the capacity of the parasite to maintain itself on these grasses, except possibly under most favorable conditions.

ISOLATION AND ARTIFICIAL CULTIVATION OF PARASITE

The isolation of the parasite, though not excessively difficult, usually can not be accomplished with as much ease as the isolation of graminicolous species of Helminthosporium generally. Plantings made on a suitable agar medium, like maize-meal agar, with small pieces of tissue dissected from the margins of growing lesions, after surface sterilization and washings in repeated changes of sterile water, while not uniformly successful, afforded the most convenient means of securing pure cultures. The fungus, on growing out of the tissue, is recognizable by the fringe of aerial mycelium, composed of filaments of relatively large, unvarying diameter, with a distinctive branching habit, and disposed in snarls of numerous and often graceful curves. The mycelium immersed in the substratum lacks this disposition, but shows a similar degree of uniformity in diameter and a similar type of branching, with the contents generally homogeneous and moderately refringent. (Fig. 1.) Transferred to fresh media, the mycelium retains these tendencies. As growth is relatively slow, even at optimum temperatures, which seem to lie between 25° and 29° C., the snarled aerial mycelium, except at the growing margin, is usually dried out and collapsed. It then appears to the naked eye as a somewhat granular or flaky white or grayish material, sprinkled

[4] The binomial under which the plantings of this grass at the Arlington Experiment Farm were recorded and under which it was reported (8) as a host of *Helminthosporium giganteum* is retained here. Specimens kindly examined by A. S. Hitchcock were referred by him to *Stipa splendens* Trin.

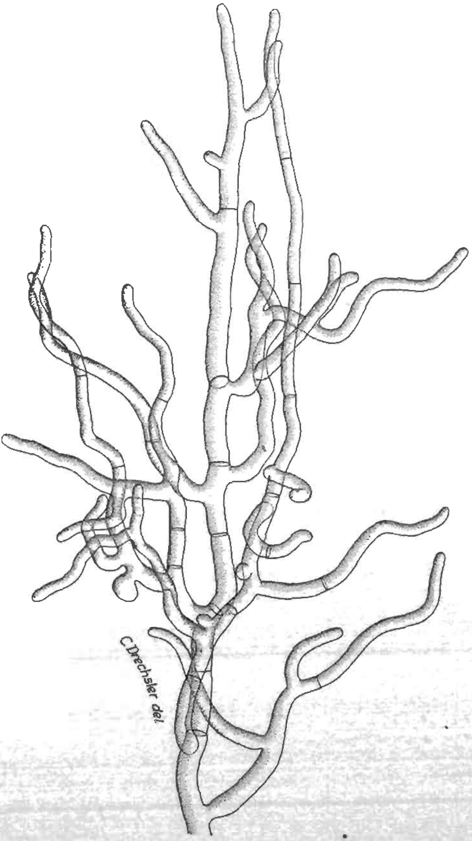


FIG. 1.—Portion of the submerged mycelium of *Helminthosporium giganteum* from the margin of growth on maize-meal agar. × 450

irregularly over the surface of the culture. (Pl. 8.) The submerged mycelium in the older portions of the culture is somewhat dark, and under the microscope appears well provided with septa, though apparently largely devoid of protoplasmic contents. A certain degree of zonation, involving both submerged and aerial mycelium, usually is evident.

Sporulation of *Helminthosporium giganteum* in culture generally is rather meager, but presents interesting features. The sporophores found scattered here and there consist of prolongations or branches of ordinary hyphae (fig. 2, D) from which they usually differ only slightly in a darker coloration and a somewhat thicker membrane. Many of the conidia are not markedly different from conidia developed under natural conditions. (Fig. 2, A–C.) Others, however, are markedly inferior in length and width. An irregular type of proliferation, evidently akin to germination, is frequent. I many instances this is expressed in the production, from the basal and apical segments where the whorl of germ tubes ordinarily arises, of two, three, or four structures that from their suggestive resemblance to conidia might be regarded as secondary conidia. (Fig. 2, C.) These may in turn become proliferous. The repetition of this process, accompanied by marked diminution in size, frequently gives rise to a ramifying system, of which the terminal elements, sometimes as little as 3.5 μ in length and 2.5 μ in diameter, are borne in short branching chains. (Fig. 3, B.) The apparatus thus produced shows marked similarities to fructifications of Hormodendron, not only in the origin of new elements by lateral and apical budding, but also in the ready disintegration of the parts. Branching systems of the same type, but without any of the larger intermediate elements resembling the conidia typical of the fungus, also are produced in some quantity directly from conidia (fig. 3, C, D) or on relatively undifferentiated mycelial branches (fig. 2, E, F; fig 3, A).

As to the possible bearing of the Hormodendronlike structures on the biology of the parasite, no information is available. So far no tendency toward proliferation other than normal germination has been observed in material collected in the field. Most of the writer's observations, however, have been made near the northern limit of the fungus, and it is not impossible that in regions of higher temperature and greater humidity the proliferous tendency may be more pronounced. In any case, regardless of its interest as a morphological detail, the Hormodendronlike development would appear to constitute a subsidiary phase resulting from a somewhat promiscuous budding process, and hence not to be compared in distinctiveness to the true conidial stage found in nature. The relationship here is comparable, perhaps, to the relationship between the widespread *Cladosporium herbarum* Link and its Hormodendron stage, which was carefully investigated by Bancroft (*1*), although the occurrence of the Hormodendron stage throughout the parasitic life of that fungus to the exclusion of the other is far from having a counterpart in the life history of *Helminthosporium giganteum*. More recently Spangler (*8*) reported the development of Hormodendron fructifications in artificial cultures of *C. fulvum* Cke. and suggested the theory that probably only one type of conidium was produced and that the two-celled bodies usually held presumptive for Cladosporium probably represent nothing but fragments of denuded conidiophores. To the extent to

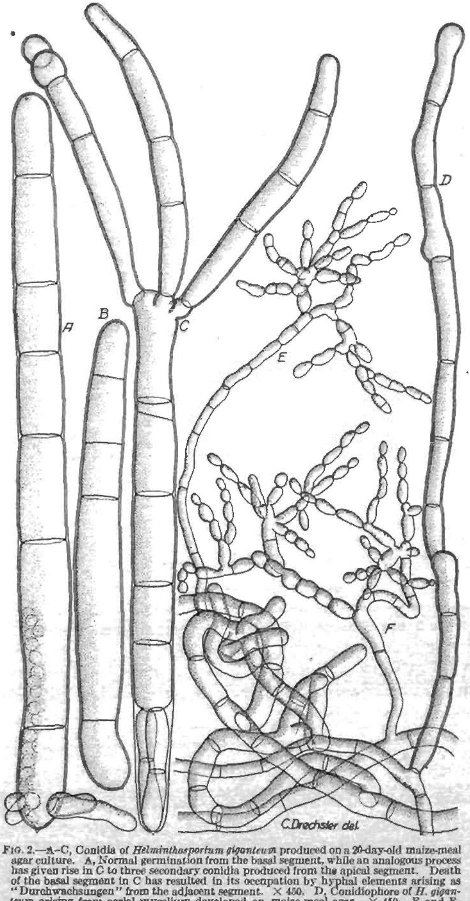


FIG. 2.—A–C, Conidia of *Helminthosporium giganteum* produced on a 20-day-old maize-meal agar culture. A, Normal germination from the basal segment, while an analogous process has given rise in C to three secondary conidia produced from the apical segment. Death of the basal segment in C has resulted in its occupation by hyphal elements arising as "Durchwachsungen" from the adjacent segment. × 450. D, Conidiophore of *H. giganteum* arising from aerial mycelium developed on maize-meal agar. × 450. E and F, Hormodendronlike fructifications of *H. giganteum* arising from aerial mycelium developed on maize-meal agar. × 450

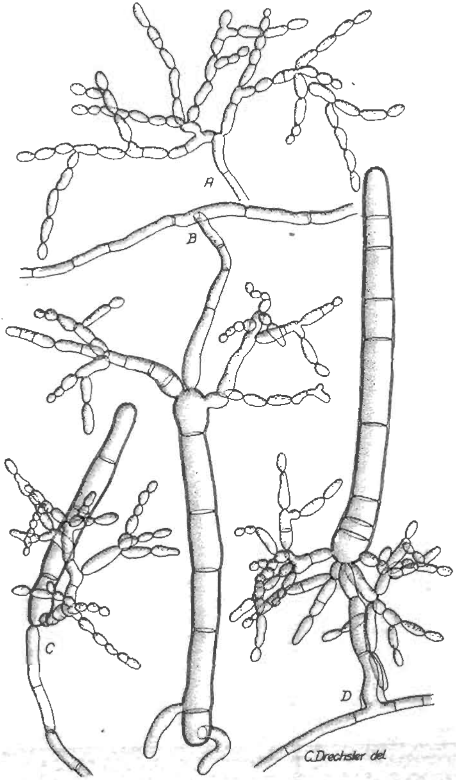


FIG. 3.—A, Hormodendronlike fructification of *Helminthosporium giganteum* arising from aerial mycelium developed on maize-meal agar. × 450. B–D, Hormodendronlike fructifications arising by proliferation from conidia of *H. giganteum* produced on maize-meal agar. × 450

which *H. giganteum* provides a parallelism, it affords little support for this view, but emphasizes rather the departures from normal morphology effected by the conditions of artificial culture. *Hormodendron hordei*, described by Bruhne (*2*) in 1894 as the cause of a leaf spot of barley (*Hordeum vulgare* L.) in Germany, is of interest in this connection as an apparently well-authenticated parasite on a gramineous host. Although the conidia from barley leaves were always warty, they gave rise to smooth spores when cultivated artificially, and the general arrangement of parts shown in Bruhne's figure (*2, Taf. 1, fig. 4*) resembles at least superficially the proliferous condition of the parasite causing zonate eyespot. Because of the presence of septate conidia, Lindau (*7, p. 700–701*) seemed inclined to regard Bruhne's fungus as a stage of Cladosporium, even though the septate structures, as in the case of *C. fulvum*, apparently were not usually terminal, and therefore might equally well have been construed as disarticulated sporophoric segments.

TAXONOMIC RELATIONSHIPS OF PARASITE

The affinities of *Helminthosporium giganteum* remain problematical. As has been pointed out previously (*5*), the large majority of graminicolous species of Helminthosporium are referable to either one or the other of two types, one having typically straight cylindrical conidia germinating indiscriminately from the intermediate as well as from the proximal and distal segments, the other with ellipsoidal conidia germinating normally by the production of two polar germ tubes. Of the species belonging to the former type, several have been identified with ascigerous conditions referable to Pyrenophora or Pleospora, and it would seem probable that a similar affinity will be found to prevail throughout. Several species of the second type have been found connected with a perfect stage, which is represented by a peculiar type of Ophiobolus characterized by helicoid ascospores. *H. giganteum* can not apparently be assigned to either category. While the conidia it produces are cylindrical, their distinctive method of germination by the production of two whorls of three or four germ tubes, one whorl arising at a little distance from the attachment and the other at an approximately equal distance from the apex, is not indicative of any close relation to the forms connected with Pyrenophora. The general appearance of the fungus in artificial culture, its slow rate of growth, the frequent disposition of the aerial mycelium in curiously curving filaments, the Hormodendronlike structure arising from hyphae or, by secondary proliferation, from conidia, the unusually regular contours and homogeneous contents of the submerged hyphae—all these attributes taken together would seem further to set off the fungus from either of the two main categories of Helminthosporium species parasitic on grasses. Although in the production of eyespot lesions the fungus is not greatly different from certain other forms, its more extensive zonate developments presents a pathological effect of striking peculiarity.

Examination of the fungus in collections of field material from different localities and various hosts gives an impression of a high degree of morphological uniformity. The limited number of strains isolated have not revealed any differences sufficiently pronounced to predominate over the rather varied expressions of cultural characters exhibited by individual strains on the same plate culture.

The occurrence of aberrant sectors in such cultures, to which some writers attach much importance, was occasionally observed. (Pl. 8.) In no instance, however, did the variants exhibit a degree of distinctiveness sufficient to make them deserving of special taxonomic consideration.

THE HOST RANGE OF THE FUNGUS

Because of its ability to infect a large variety of hosts, and because of the wide range in degree of pathogenicity expressed, from the production of a barely discernible lesion to the almost complete destruction of the foliage of plants attacked, *Helminthosporium giganteum* might well serve as a subject for inquiry into the intimate aspects of parasitism. Owing to the difficulty of obtaining conidia of the parasite in artificial culture, however, greenhouse experimentation following the usual methods might not be easy of accomplishment. In the absence of such experimentation, field observations on mixed stands of grasses, including one or more species upon which the fungus sporulates abundantly, may not be devoid of interest. Some observations of this kind, together with descriptive data, are presented in the following paragraphs.

Agropyron repens mixed with heavily infected *Cynodon dactylon* was found severely attacked in various localities in the vicinity of the District of Columbia. Equally severe infections were observed, however, in a number of situations where no admixture of Bermuda grass was present, thus supplying proof of a degree of congeniality high enough to permit the fungus to propagate itself luxuriantly independent of other hosts. The individual lesions (pl. 2, I–M) do not generally exceed 1 mm. in width and 3 to 5 mm. in length, although sometimes the latter dimension may approximate 8 mm. They are straw colored in the center and delimited by a narrow dark-brown marginal zone. The zonate type of development usually may be observed, although the destruction of leaves more often is attributable to the abundance of moderate-sized lesions, several hundred of which not infrequently may be present on an individual foliar organ. After the death of severely infected leaves a liberal production of sporophores and spores ensues, the former arising not only from the discolored areas but also from the surrounding tissue.

The abundance of *Agropyron repens*, together with its high degree of susceptibility, seems to indicate that this grass might become the most important host of *Helminthosporium giganteum* in sections near the northern range of the parasite wherever Bermuda grass is present only in lesser quantity.

In August, 1922, at the Arlington Experiment Farm, Rosslyn, Va., *Agropyron elongatum* was found affected with *Helminthosporium giganteum*, although somewhat less severely than *A. repens*. While the infections resulting directly from germinating conidia appeared in considerable number, the lesions remained mostly of small dimensions. (Pl. 2, A.) When secondary enlargement took place and groups of lesions became confluent (pl. 2, B C), more severe effects were brought about. In the same plot another congeneric host, *A. intermedium*, revealed infection of somewhat less severity than that prevailing in quack grass, though otherwise not dissimilar. (Pl. 2, D–H.)

Agrostis stolonifera L., in September, 1922, at Arlington Experiment Farm, showed elliptical spots approximately 1 mm. in width and up to 2 or 3 mm. in length, which were readily attributed to conidia of *Helminthosporium giganteum*. The affected areas, for the most part almost white, were delimited from the healthy tissue by a very narrow, inconspicuous dark-brown margin. (Pl. 2, N–Q.) Owing, apparently, to the small size of the leaves, when a number of lesions occurred on the same blade, withering of the parts more distal in position resulted, although the entire damage caused was not excessive. No extensive zonate development was observed. As the eyespot lesions for the most part remained free from conidiophores of the parasite, it is not evident that the latter is capable of maintaining itself on creeping bent grass.[5]

Bromus inermis was found severely attacked by *Helminthosporium giganteum* at Arlington Experiment Farm during the season of 1922. The number of eyespot lesions, to be sure, was not excessive. In the absence of secondary development they generally did not attain immoderate size, those measuring more than 1 mm. in width and 2 or 3 mm. in length, including the deep-brown delimiting margins, being rather exceptional. (Pl. 3, A–D.) Secondary development, however, was relatively frequent and often extensive. (Pl. 3, D.) On the large regions of killed tissue, sporulation took place abundantly. Undoubtedly awnless brome grass may be regarded as more subject to damage than most of the several cultivated grasses included among the various hosts discussed in the present account. It may be a fortunate circumstance, therefore, that the area over which it is being grown for forage lies well north of the latitudes in which the fungus has hitherto been observed.

Chaetochloa lutescens, although often found growing in proximity to heavily infected Bermuda grass in the vicinity of the District of Columbia during the season of 1922, never exhibited any lesions due to *Helminthosporium giganteum* in any of the collections made in that general locality. That the grass is nevertheless not entirely immune from infection is evident in the occurrence of eyespot lesions in material collected near Hurlock, Md., in August, 1923, as well as in a collection made near Kennett, Mo., in August, 1924. In both cases the source of the infecting conidia was badly diseased Bermuda grass, in a stand of which the yellow foxtail grass occurred as an intimate admixture. The lesions, so few in number as almost to escape detection, were of relatively small size, not usually exceeding 1 mm. in length and 0.5 mm. in width, and of an elliptical shape, with a

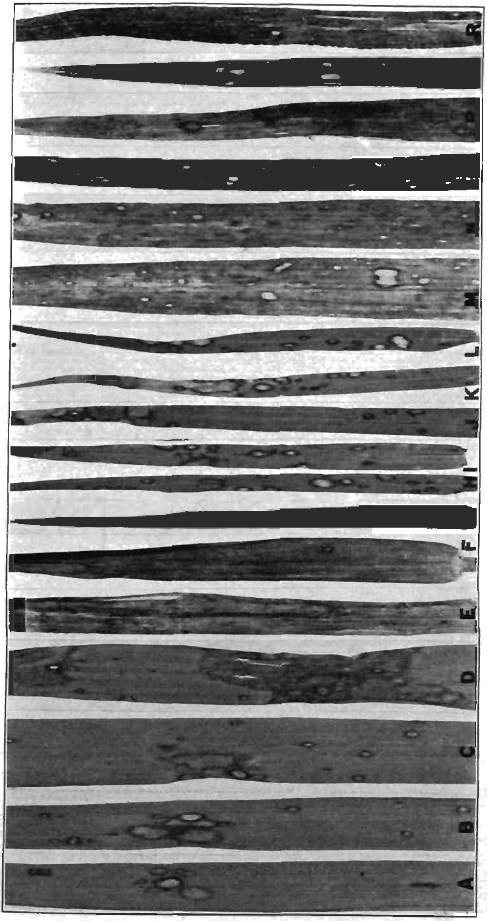
[5] Severe infection of creeping bent was noted in some of the turf plots and nursery rows at the Arlington Experiment Farm during the season of 1928, the very evident destructiveness of the parasite even in the absence of artificial watering being associated with ready centrifugal development of lesions and abundant sporulation. The position of the grass under consideration as an independent host was confirmed in a striking way by the occurrence of zonate eyespot in putting greens planted with it. This was true not only in regions generally favorable for the development of the fungus, but also, as has been mentioned in another connection, in territory not known to harbor the parasite on any host under ordinary conditions. In the vicinity of La Fayette, Ind., greens visited by the writer on Aug. 28, 1928, showed heavy infection, though perhaps owing to cooler conditions the infection then was less severe than that represented in specimens collected from the same grounds on July 20, 1928, by A. A. Hansen. Golf courses in the vicinity of Detroit, Mich., visited Sept. 1, 1928, showed the parasite active on some greens, though only in moderate or even small quantity; and a similar degree of prevalence was found also at Wooster, Ohio, visited Sept. 4, 1928. Specimens originating from near London, Ohio, from near De Kalb, Ill., from near Highland Park, Ill., and from near Minneapolis, Minn., in August and September, 1928, provide additional testimony of the efficacy and wide distribution of *Helminthosporium giganteum* as a turf parasite. It may be mentioned that not all strains of creeping bent are attacked with equal severity, some strains appearing almost completely resistant. Indeed the meager infection observed at the Arlington Experiment Farm in 1922 is to be explained by the fact that during that season only resistant types were represented in the nursery rows, whereas the destructive infection recorded for 1928 was limited to one or several very susceptible types subsequently added to the plantings.

straw-colored central portion surrounded by a brownish margin. (Pl. 3, E, F.) As might be expected, conidiophores of the parasite were never observed on any of the spots.

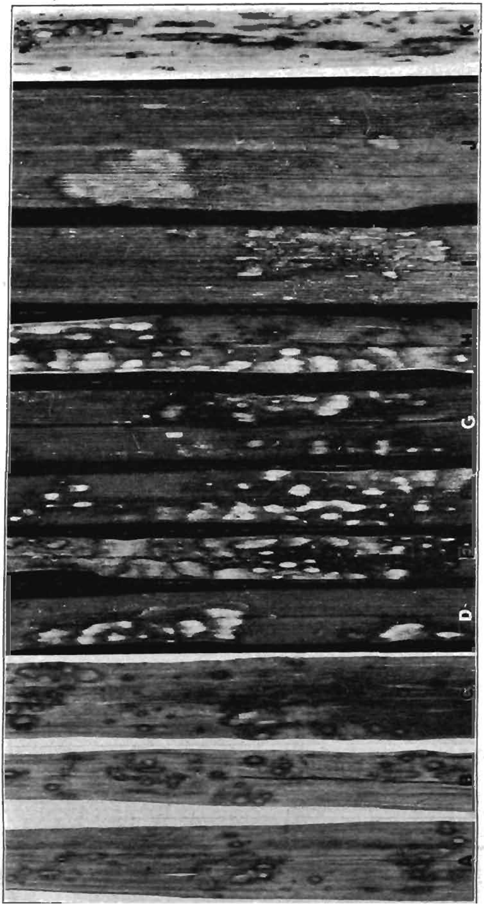
Cynodon dactylon unquestionably serves as the principal host of *Helminthosporium giganteum* in the United States. Infection by germinating conidia evidently takes place with unusual readiness, the foliage usually becoming spotted with lesions of independent origin much more abundantly than is shown in Plate 3, G–I. When conditions are favorable, the secondary type of development occurs (pl. 3, J–L) in about the same measure as in *Agropyron repens* and *A. intermedium*, and therefore perhaps somewhat less extensively than in *Phalaris arundinacea* or even in *Bromus inermis* and *Eleusine indica*. Nevertheless, because of its widespread distribution throughout at least the more favorable range of the fungus, and the readiness with which sporulation proceeds on the diseased foliage, mostly on tissue involved in the coalescence of groups of crowded individual lesions, Bermuda grass appears, on the whole, to support the parasite in as large quantity as all the other hosts taken together. The densely massed habit it frequently adopts when left undisturbed on suitable soil seems to be unusually favorable for the development of the fungus, so that the more luxuriant stands are frequently all but killed outright, the severity of such attack not being exceeded by any foliar grass disease known to the writer.

Digitaria humifusa Pers. (*Syntherisma ischaenum* Schrad. Nash), growing mixed with heavily infected Bermuda grass at Kennett, Mo., in August, 1924, bore a liberal sprinkling of lesions due to *Helminthosporium giganteum*. These lesions (pl. 3, M–O) occurred as elliptical spots rarely exceeding 2 mm. in length and 1 mm. in width, and having a straw-colored center with a reddish brown delimiting margin. The zonate type of development never was manifested. Examination of the eyespot lesions failed to reveal any conidiophores of the parasite in question. In the same location *Digitaria sanguinalis* bore eyespot lesions caused by germinating conidia of *H. giganteum*, resembling those borne on the congeneric host, but exhibiting a somewhat broader, more deeply colored border, and occurring so sparingly that their discovery entailed considerable search. The leaf shown in Plate 3, Q, was very unusual, in that nearly a dozen spots were found relatively close together, while that shown in Plate 3, R, with only two, also represents a more heavily infected condition than obtained generally. No conidiophores were found on any of the lesions on crabgrass.

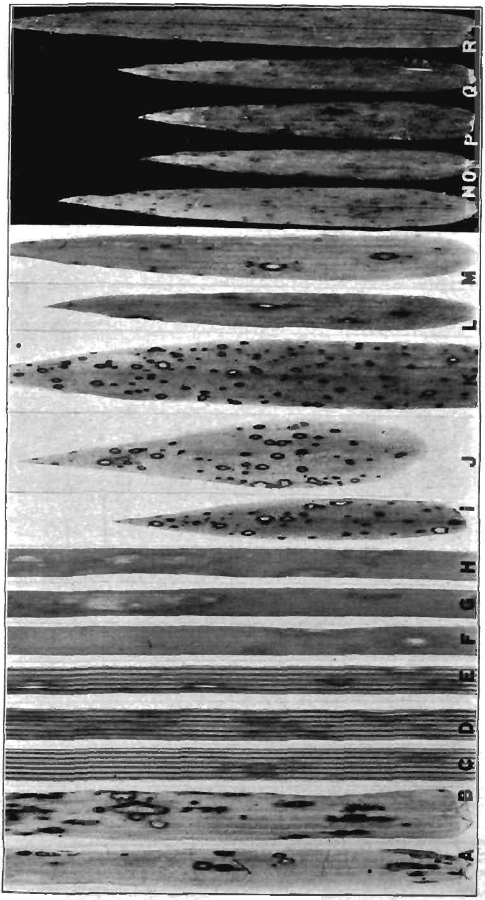
Echinochloa crusgalli was found attacked by *Helminthosporium giganteum* near Kennett, Mo., in August, 1924. It exhibited a considerable degree of susceptibility, the individual lesions being not only fairly numerous but also often showing moderately extensive secondary development. (Pl. 4, A–C.) Reddish brown coloration was somewhat conspicuous, being present on the relatively broad margins delimiting the discrete lesions, as well as in larger blotches encompassing areas killed as a result of secondary development, or resulting from coalescence of a number of separate spots. On the larger withered parts sporophores and spores were being produced in quantity. Barnyard grass would seem to show sufficient congeniality to serve as one of the more important hosts, although in the localities



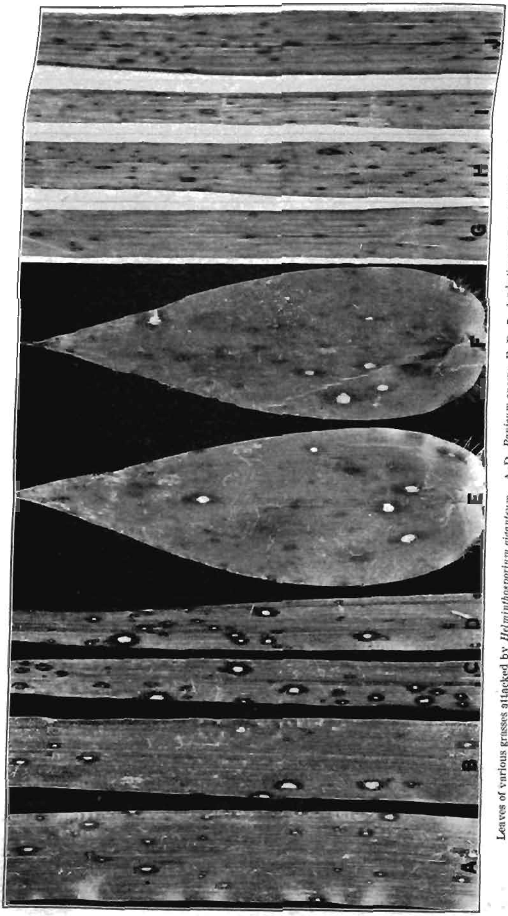
Leaves of various grasses attacked by *Helminthosporium giganteum*. A–D, *Bromus inermis*; E, F, *Chaetochloa lutescens*; G–L, *Cynodon dactylon*; M–O, *Digitaria humifusa*; P–R, *Digitaria sanguinalis*. ×2



Leaves of various grasses attacked by *Helminthosporium giganteum*. A–C, *Echinochloa crusgalli*; D–H, *Eleusine indica*; I, J, *Elymus virginicus*; K, *Eragrostis major* ×2



Leaves of various grasses attacked by *Helminthosporium giganteum*. A, B, *Eragrostis major*; C–H, *Lasiagrostis splendens*; I–K, *Leersia virginica*; L, M, *Muhlenbergia mexicana*; N–R, *M. schreberi*. ×2



Leaves of various grasses attacked by *Helminthosporium giganteum*. A-D, *Panicum anceps*; E, F, *P. clandestinum*; G-J, *P. dichotomoflorum*. × 2

in which the writer made his observations it did not occur abundantly enough to play any large part in the maintenance of the parasite.

Eleusine indica is to be included among the grasses most susceptible to *Helminthosporium giganteum*. In infected stands lesions originating from separate infections may become so numerous as to coalesce and thus lead to the withering of individual leaves, or considerable portions of a leaf may be killed directly as a result of secondary enlargement of some of the lesions. (Pl. 4, D–H.) Reddish brown coloration is present in the narrow marginal zones delimiting the individual lesions and also in less sharply localized form in markings on the larger affected parts. Sporulation under suitable conditions is abundant. Goose grass has been found more or less seriously affected wherever the parasite has been encountered. Owing to its general distribution throughout the known range of the parasite, its importance as a host of *H. giganteum* would seem second only to Bermuda grass. Like the latter, it frequently serves as the source from which other grasses in close proximity become infected.

Elymus virginicus, growing at Arlington Experiment Farm within 10 meters of diseased Bermuda grass in the season of 1922, showed a somewhat unusual condition relative to its infection by *Helminthosporium giganteum*. The individual lesions resulting directly from germinating conidia were few in number, but a large proportion of these showed extensive secondary development. (Pl. 4, I, J.) On the zonate areas of killed tissue sporophores were produced abundantly. The appearance suggested that infection of the coarse foliage by germinating conidia was attended with difficulty, but that once the parasite gained a foothold its further development centripetally took place readily. Under suitable conditions the grass would seem to be capable of serving as a congenial host.

Eragrostis major, growing in mixed stand with very heavily infected Bermuda grass near Seat Pleasant, Md., in September, 1922, exhibited meager infection by *Helminthosporium giganteum*. The large majority of lesions were of the eyespot type, elliptical in shape, from 0.2 to 0.8 mm. in width and 0.4 to 1.6 mm. in length, with a rather conspicuous deep reddish brown marginal zone surrounding a central bleached area usually of minute size. (Pl. 4, K; 5, A, B.) In a number of instances, however, secondary development had resulted in the death of more extensive portions of tissue measuring sometimes from 10 to 20 mm. in length and from 2 to 3 mm. in width, or even extending entirely across the leaf. Withering of the distal portions of certain foliar organs in some cases appeared to result from such more extensive development of the parasite, or from an unusual concentration of smaller lesions, although a certain degree of doubt as to the causal relation of the parasite was introduced because of the approaching maturity of the host. Sporophores of the fungus were found on the larger lesions and on withered parts bearing numbers of smaller spots in close proximity to one another. Such reproduction, however, was on a decidedly small scale, and it remains somewhat uncertain, therefore, whether the fungus could propagate itself successfully on stink grass alone.

Lasiagrostis splendens, growing at some distance from heavily infected reed canary grass at Arlington Experiment Farm in 1922, became severely infected with *Helminthosporium giganteum*. Individual lesions were fairly numerous, the smallest ones appearing as

uniformly dark-brown blotches. (Pl. 5, C. D.) Those of intermediate size, measuring 2 to 4 mm. in length, were generally of the simple eyespot type (pl. 5, E. F), while the more extensive morbid areas, frequently exceeding 1 cm. in length and including the entire width of the leaf, bore the zonate markings characteristic of secondary development. (Pl. 5, G, H.) On the latter type of lesion sporophores and spores of the parasite were found occurring in abundance. Because of the strong dorsiventral differentiation between the prominently veined dark-green upper surface of the foliage (pl. 5, C–E) and the smoother, lighter green under surface (pl. 5, F–H) the two aspects of the lesions appear different to a rather unusual degree. The fungus would seem capable under suitable conditions of causing more than appreciable injury to the grass and unquestionably could maintain itself thereon independent of other hosts.

Stands of *Leersia virginica*, occurring in close proximity to heavily infected Bermuda grass or quack grass at various points along the Chesapeake & Obio Canal, have regularly become thickly peppered with numerous eyespot lesions during the five seasons in which observations were continued. While the spots usually remain relatively small, rarely exceeding 2 mm. in length and 1 mm. in width (pl. 5, I–K), they occasionally become confluent, and thus bring about the death of somewhat larger portions of leaf. Even these larger areas, however, are usually devoid of conidiophores of *Helminthosporium giganteum*, although in somewhat exceptional instances a very sparse array of such structures has been observed. That such meager sporulation, nevertheless, is not entirely without significance became evident through the discovery in September, 1922, of a pure solitary stand of white rice grass on which an infection with *H. giganteum* occurred obviously quite independent of other hosts. As this stand was situated on a large fill on which other grasses had not encroached, it was not difficult to verify the absence of external sources of infection within a radius of more than 50 meters. It is interesting to note that extensive secondary development of the fungus from relatively few lesions, rarely observed elsewhere, here accounted largely for the injury observed, which, to be sure, was inconsiderable. Sporulation on the larger tracts of leaf tissue involved in such development was only slightly more abundant than on the leaves bearing the numerous small lesions of independent origin. The grass is to be regarded, perhaps, as hardly a more congenial host than *Eragrostis major*, even though under exceptional conditions it permits autonomous propagation of the parasite.

Sometimes *Leersia virginica* can be found attacked simultaneously by both *Helminthosporium giganteum* and *H. leersii* Atk. As the older lesions caused by the latter fungus are many times larger than eyespot lesions due to the former, and never exhibit the zonate markings characteristic of the secondary development of *H. giganteum*, their identification usually entails little trouble. The smaller lesions of *H. leersii*, also, can generally be distinguished from those of *H. giganteum* because of their broader and less sharply defined marginal zone. In doubtful instances microscopic examination is necessary. Since neither fungus sporulates on any except the largest regions of affected tissue, identification of smaller spots is most conveniently

accomplished by determining the presence or absence of the evacuated spore membrane of *H. giganteum*.

Muhlenbergia mexicana, at a distance of about 5 meters from heavily infected quack grass, revealed relatively few scattering lesions of *Helminthosporium giganteum*. The larger ones were elongated elliptical in shape, measuring 2 to 3 mm. in length by 0.5 mm. in width, and showing sharp differentiation between the small central bleached portion and the narrow dark-brown delimiting zone. (Pl. 5, L, M.) Most of the lesions were of the unmodified eyespot type and quite devoid of sporophores of the parasite. Occasionally the presence of minute specklike discolorations in zonal arrangement about one of the larger lesions evidenced a somewhat meager secondary development. In certain of the largest lesions sporophores of the fungus were observed, although the total production of such structures was so small that it is to be doubted whether the fungus could persist on *Muhlenbergia mexicana* in the absence of more favorable hosts, except under very favorable conditions.

Muhlenbergia schreberi, in the same locality as *M. mexicana* but occurring in intimately mixed stand with heavily infected quack grass and goose grass, exhibited lesions of *Helminthosporium giganteum* in moderate number. These lesions were characterized by unusually small size, sharp definition of the margin from the healthy tissue and the bleaching of the center in spite of relatively minute proportions. (Pl. 5, N–R.) No extensive secondary development or evidence of sporulation ever was observed on this grass, which evidently does not permit autonomous development of the fungus. Owing to the frequent occurrence, on the more mature foliage, of numerous minute dark linear lesions somewhat resembling those due to conidia of the parasite under consideration, but associated with another fungus, spots not bleached in the center can not be identified without microscopic inspection.

Panicum anceps Michx., occurring interspersed in a stand of heavily infected quack grass during the season of 1922, exhibited eyespot lesions in moderate quantity. These lesions sometimes attained a length of 3 to 4 mm. and a width of 1.5 to 2 mm., although usually their proportions did not exceed one-half of the values mentioned. (Pl. 6, A–D.) They exhibited a bleached center on attaining a length of 1 mm., the delimiting margin being usually relatively broad and light brown in coloration, rather than dark brown. As none of the lesions were found bearing conidiophores of *Helminthosporium giganteum*, the grass can not be considered among the congenial hosts.

Panicum clandestinum, because of its habit of occupying the weedy borders of neglected fields, which, after the middle of summer in the vicinity of the District of Columbia, are often overrun with Bermuda grass, was frequently found exposed to infection from the great profusion of conidia produced by *Helminthosporium giganteum* on the latter host. The foliage then exhibited discoloration in the form of numerous dark-brown specks, or of larger nebulous blotches, or of well-defined eyespot figures, with a relatively wide, vaguely delimited marginal zone. (Pl. 6, E–F.) When the younger leaves thus affected were examined microscopically, these discolored portions could in all instances be found associated with collapsed remains of overlying conidia of the parasite. Although the larger eyespot lesions

contained bleached central areas that sometimes measured 4 mm. in length by 1.5 mm. in width, sporophores of the fungus never were observed. In spite of a relatively high degree of susceptibility to conidial infection, the grass is apparently not sufficiently congenial as a host to sustain *H. giganteum* independently.

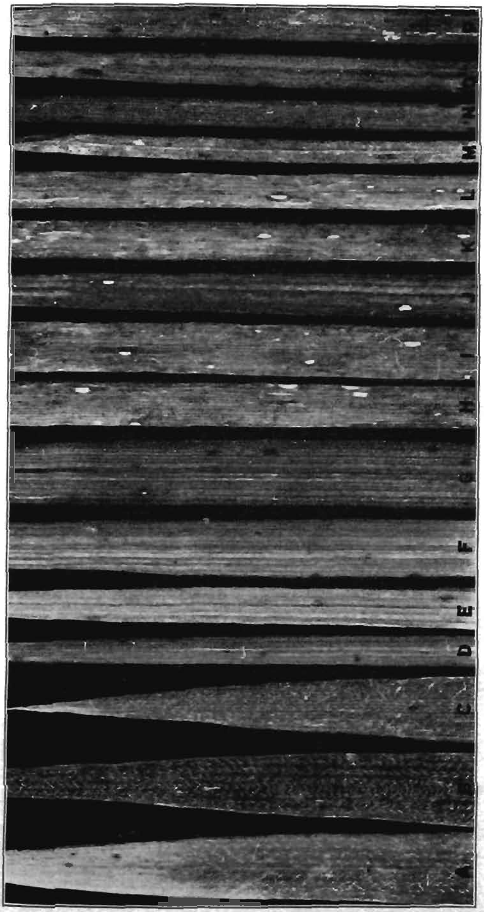
Pancium dichotomoflorum was found intermixed with infected goose grass in a number of truck fields and vegetable gardens near the District of Columbia in September, 1922. Many of the leaves thus exposed bore a varying number of lesions from infection by conidia of *Helminthosporium giganteum*. Near Kennett, Mo., where, in September, 1924, the grass occurred in mixed stand with heavily infected Bermuda grass, a considerably more abundant infection obtained. In both localities the lesions were represented by reddish-brown or dark-brown spots, rather sharply defined from the healthy tissue, somewhat linear or streaklike in outline, variable in size, often being so small as to be barely discernible, but sometimes attaining a length of 3 mm. and a width of 1 mm. (Pl. 6, G–J.) Many of the lesions were bleached in the center, but in other instances this feature was not evident. None of the material from either source revealed the presence of sporophores.

Panicum gattingeri, growing in a stand of infected quack grass at Cabin John, Md., in September, 1922, bore a meager sprinkling of eyespot lesions due to infection from conidia of *Helminthosporium giganteum*. The spots were small in size, being not more than 1 mm. long and less than half as wide, yet usually exhibiting a bleached center. (Pl. 7, A–C.) Conidiophores of the fungus never were observed. The grass is evidently considerably less susceptible to infection than any of the three congeneric species mentioned, and is to be included among the more unfavorable hosts.

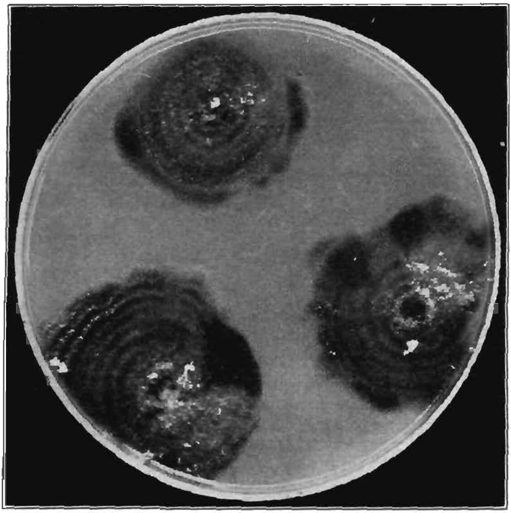
Pennisetum alopecuroides (L.) Spreng.,[6] growing at a distance of less than 1 meter from heavily infected reed canary grass at Arlington Experiment Farm in 1922, showed on some leaves scattered lesions due to infection from conidia of *Helminthosporium giganteum*. These lesions were present generally as reddish brown blotches, although a few were of the eyespot type, with the bleached center sharply defined. (Pl. 7, D–G.) None ever revealed the presence of conidiophores. In view of the quantity of inoculum to which the foliage was exposed throughout the season and the insignificance of the injury occasioned, the grass would appear to possess little susceptibility to attack by the fungus.

The extreme congeniality of *Phalaris arundinacea* as a host of *Helminthosporium giganteum* has been discussed in another connection. It must be mentioned, however, that the parasite has not been encountered on reed canary grass elsewhere than in the plots at Arlington Experiment Farm. Several wild stands observed in the vicinity of the District of Columbia never revealed any sign of infection, even during the very favorable season of 1922. As all of these stands have happened to occur in isolated situations, separated from infected grasses by wooded terrain, the absence of the fungus was not difficult to explain. Nor have infections ever been observed

[6] This host was reported previously under the binomial *Pennisetum japonicum* Trin. The change in specific name is made on the advice of Agnes Chase, who kindly examined specimens and identified them as clearly belonging to the species frequently designated as *P. compressum* R. Br. The plant is of course conspicuously different from pearl millet (*P. glaucum* (L.) R. Br.), to which it would appear the term *alopecuroides* during a long period of nomenclatorial confusion has often been erroneously applied.



Leaves of various grasses attacked by *Helminthosporium giganteum*. A-C, *Panicum gattingeri;* D-G, *Pennisetum alopecuroides;* H-L, *Phleum pratense;* M-P, *Poa pratensis.* ×2



Maize-meal agar plate culture of *Helminthosporium giganteum* 15 days after inoculation. × 1

on the ornamental form known as ribbon grass, *Phalaris arundinacea* var. *picta* L.

During several seasons *Phleum pratense*, found growing among heavily infected Bermuda grass and quack grass in a number of locations near the District of Columbia, exhibited, in meager number, lesions caused by germinating conidia of *Helminthosporium giganteum*. These lesions usually were relatively small, rarely exceeding 2 mm. in length or 1 mm. in width. (Pl. 7, H–L.) Often they consisted only of bleached portions of tissue, thus appearing as white spots, devoid of any colored margin, while in other cases a narrow brown border was recognizable. The scarcity of infection and the complete absence of conidiophores from the relatively small diseased parts denote a high degree of resistance to the parasite.

Poa pratensis, often found exposed to infection in the same situations as timothy, exhibited approximately the same low degree of suceptibility. The lesions were similarly few in number and of equally small dimensions, some, indeed, being so minute as to be barely discernible. (Pl. 7, M–P.) A conspicuous difference was represented in the dark-brown or brownish-black color of the spots on Kentucky bluegrass. The bleached center distinctive of the eyespot lesions was present only in exceptional instances, and then somewhat vaguely, most of the discoloration appearing in the form of unrelieved elongated or almost linear specks.

SUMMARY

Helminthosporium giganteum occurs generally throughout the Southern States and has been found in quantity as far north as Maryland and Missouri. In the vicinity of the District of Columbia the conidia from centers of infection do not appear to spread beyond distances of 20 meters in one season, a limitation due apparently to the large size and short period of viability of these structures, and to which may be attributed, in part at least, the frequent irregularities noticeable in the local distribution of the parasite. The fungus seems to overwinter in the form of dormant mycelium, fresh conidiophores and conidia being produced in late spring from the morbid parts of old foliage infected during the previous season.

Sporulation of the fungus occurs on the larger tracts of killed tissue, resulting either from the coalescence of numbers of individual eyespot lesions or from secondary development of such lesions. The latter type of development occurs only when the leaf surface is coated with moisture, and involves the production, centrifugally from the margin of the morbid tissue, of a prostrate mycelium that in occupying the surrounding parts brings about a water-soaked condition and later desiccation and death. As in various hosts the newly infected parts are delimited by marginal coloration, the repetition of such development brings about a characteristic zonate appearance.

Among the hosts on which sporulation was observed under natural conditions, and on which the parasite apparently could propagate itself, are to be included *Agropyron elongatum*, *A. intermedium*, *A. repens*, *Bromus inermis*, *Cynodon dactylon*, *Eleusine indica*, *Echinochloa crusgalli*, *Elymus virginicus*, *Lasiagrostis splendens*, *Leersia virginica*, and *Phalaris arundinacea*. Sporulation was observed also, though in meager quantity, on *Eragrostis major* and *Muhlenbergia mexicana*. Lesions due to infection by conidia of the parasite were

observed on *Argostis stolonifera*, *Chaetochloa lutescens*, *Digitaria humifusa*, *Muhlenbergia schreberi*, *Panicum anceps*, *P. clandestinum*, *P. dichotomoflorum*, *P. gattingeri*, *Pennisetum alopecuroides*, *Phleum pratense*, and *Poa pratensis* when these grasses occurred in proximity to more congenial hosts.

When grown in pure culture on artificial media the fungus develops relatively slowly. Meager and somewhat abnormal sporulation generally takes place, the conidia as well as the mycelial hyphae often giving rise to branching systems of small disarticulating elements, the whole closely resembling the fructifications usually referred to the form genus Hormodendron. This proliferous phase, and more especially the distinctive method of germination by the production of two whorls of germ tubes, one from near each end of the conidium, would seem to set the fungus apart from the two most numerous series of graminicolous forms included in the genus Helminthosporium.

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STUDIES ON FIRE BLIGHT: HOST RANGE[1]

By H. R. Rosen, *Associate Plant Pathologist*, and A. B. Groves, *formerly Graduate Assistant in Plant Pathology, University of Arkansas*

INTRODUCTION

For the past 50 years or more the disease of rosaceous plants popularly known as fire blight has been the subject of innumerable studies, mainly by American plant pathologists. Over 200 articles on fire blight have recently been reviewed by the junior author (*10*),[2] every one of which added some facts that had not been known prior to its publication. Hundreds of other articles and bulletins on the same subject were excluded from this list because they represented no new work and were largely written for popular reading. In spite of this impressive array of literature it can hardly be said that practical and economical measures for the control of this disease have yet been devised. Aside from the practical aspects, many fundamentally important scientific facts are wanting. Even the morphology of the pathogene, a question which is apt to receive the first attention in the study of any parasitic disease, has not been fully established. Discussions of cultural and physiological reactions of the pathogene are often conflicting and wanting in exact details, many of them having been written at a time when the science of bacteriology was very young (*3*), and when pure cultures were frequently not obtained, owing to the imperfect and laborious technic then available. Does the organism overwinter only in very susceptible varieties and species or is it free from such limitations? Why are plants extremely susceptible in one month and very resistant in the month following? By what means does the organism produce the disease? These questions have not received adequate attention.

THE RANGE OF HOSTS

From the standpoint of parasitism in general and cellular pathology in particular, the disease producer here involved offers some exceptionally tangible points of investigation. Very few bacterial pathogenes are capable of attacking such a wide range of genera, species, and varieties of plants. Two new genera and several new species of hosts will here be presented, and there can be little doubt that others remain to be discovered. Now, while most of the bacterial pathogenes capable of attacking a wide range of hosts are primarily wound parasites, such as *Bacterium tumefaciens* and *Bacillus carotovorus*, *B. amylovorus*, on the other hand, is fully capable of entering through at least one kind of natural opening, the nectary, and perhaps under certain conditions, if we accept Heald's work (*11*), it may also enter through the hydathodes. (Brooks's recent investigations (*6*) seems to throw some doubt on the last possibility, and there can be little

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[2] Reference is made by number (italic) to "Literature cited," p. 504.

doubt that aside from blossom infection, most, if not all, of the others, including those on leaves, fruits, twigs, large limbs, and trunks, as well as roots, involve infections through wounds.) Here then is a pathogene capable of infecting through natural openings as well as through wounds and at the same time possessing a rather wide host range. These facts, as already intimated, open up certain interesting possibilities concerning parasitism. If chemotaxy plays any rôle at all in initiating infections in this disease it must be dependent upon one or more substances which are common to all the hosts involved. Then again the process involved in the production of plasmolysis, cellular discoloration, necrosis, and disintegration by this organism, in brief the manner in which it produces disease on all the hosts, can not easily be looked upon as due to any one toxic substance eleborated by the parasite which possesses any marked degree of specificity but may more reasonably be sought in some general process in which normal cellular functions are interfered with. Aside from these questions engendered by a knowledge of the proper host range, it is very important to know all the susceptible species of plants because of the part which any one plant may have in carrying the disease producer through the winter or because such plants may be important disseminating centers during the growing season.

ARTIFICIAL INFECTIONS ON JAPANESE QUINCE

While many references can be found in the literature to natural and artificial infections on the cultivated quince, *Cydonia oblonga* Mill. (*C. vulgaris* Pers., *Pyrus cydonia* L.), no reference has been found to natural or artificial infections on the flowering quince, *Chaenomeles lagenaria* Koidz. (*C. japonica*-Hort., *Cydonia japonica* Hort.), a shrub very commonly used for ornamental purposes over a large part of the United States. As this plant is considered to be closely related to the quince, being placed in the genus Cydonia by some taxonomists and in the closely related genus Chaenomeles by others, it seemed desirable to ascertain its susceptibility to the fire-blight organism. In a preliminary experiment begun on March 29, 1928, a number of blossoms attached to a flowering quince plant growing out of doors were sprayed with a pure culture of *Bacillus amylovorus* which had been isolated from a blighted apple twig and which when inoculated into potted Bartlett pear plants growing in a greenhouse had proved to be very virulent. By April 10 a few of the inoculated blossoms showed various signs of blight, including a withering and discoloration of the petals and a dark green water-soaked appearance of the receptacles. Microscopic examination of the receptacles showed the tissues to be teeming with bacteria of a size and shape which clearly indicated the fire-blight bacillus. Uninoculated blossoms appeared perfectly sound and without any evidence of infection. As it is difficult to maintain proper controls out of doors it was decided to repeat the experiment in the greenhouse in a more adequate fashion. A number of shoots were cut off, some bearing blossoms and others representing newly developed leafy twigs, and placed with their cut ends in vessels containing water.

On April 10, 59 blossoms were inoculated, 31 by spraying with a broth suspension of the strain previously mentioned and 28 by

injecting the receptacles with a hypodermic needle. The control consisted of 45 blossoms borne on twigs which were kept apart from those inoculated with the bacterium. Of this number 18 were injected with sterile water by means of a hypodermic needle and 27 were sprayed with sterile water. Three days later a considerable number of the inoculated blooms showed clear signs of blight similar to those noted in the out-of-door inoculations. On April 19, 26 of the 28 plants inoculated with the needle had blighted and 26 of the

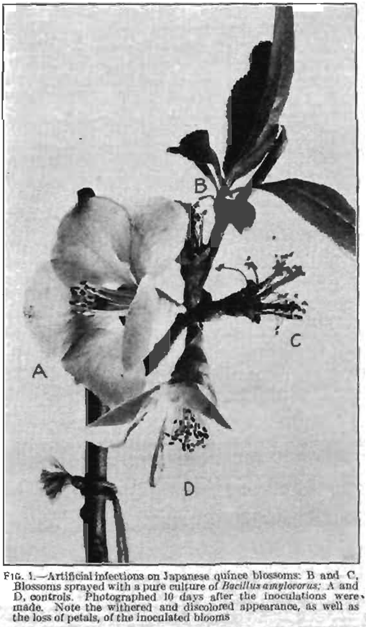

FIG. 1.—Artificial infections on Japanese quince blossoms: B and C, Blossoms sprayed with a pure culture of *Bacillus amylovorus;* A and D, controls. Photographed 10 days after the inoculations were made. Note the withered and discolored appearance, as well as the loss of petals, of the inoculated blooms

31 sprayed plants showed unmistakable symptoms of blight. (See fig. 1.) All the control blossoms remained healthy. In spite of the fact that the blooms blighted so readily, in no case was the disease found to extend into the older tissues of the subtending twigs, being entirely confined to the blooms, and giving them the appearance of having been injured by frost. Indeed the resemblance to frost injury is so striking that it would be very difficult to distinguish one from the other without microscopic examination. Typical blight-

producing bacteria were again observed in the diseased blossoms, being found in the petals, calyx, receptacle, and ovary. One of these blighted blooms was carefully washed, the surface sterilized with mercuric chloride, macerated, and used for a series of poured plates. From these the organism was recovered in pure culture and when inoculated into healthy pear shoots of vigorously growing Bartlett plants maintained in the greenhouse, typical blight was produced.

Since no twig blight had developed as a result of the blossom infections, and since the blossoms in this plant are carried on old wood, it seemed worth while to determine the susceptibility to the disease of young, newly developed, leafy shoots. For this purpose a number of young and succulent twigs were inoculated hypodermically and a similar number, used as controls, were injected with sterile water. Here, as in the blossom inoculations, infections occurred very readily. (See fig. 2.) Within a week after the inoculation the disease ex-

FIG. 2.—Artificial infections on Japanese quince twigs. The four upper shoots were inoculated with a pure culture by the use of a hypodermic needle. The lower shoots, serving as controls were injected with sterile water. *Photographed eight days after the inoculations were made*

tended along several inches of the twig, causing it to wither and become dark brown and droopy, killing the attached leaves. Altogether, the twig infections appeared very similar to those observed in ordinary blight of apply twigs, except that the injured and dead twigs showed a greater tendency to droop than do blighted apple twigs. The disease, having shown a progressive killing of adjoining tissues for about 15 days following the first symptoms, soon ceased to extend any further and became sharply delimited by a well-defined margin which frequently appeared depressed in contrast to the adjoining healthy tissues. The dead leaves showed the same tendency to hanging on to the twigs for considerable periods that is often manifested by other hosts. The microscopic observations of the diseased twig tissues revealed the typical blight-producing organisms, and the reisolations, carried out as in the artificial infections of the blossoms, resulted in pure cultures of virulent, blight-producing bacteria, as proved by inoculations on growing pear shoots. The controls all remained sound and free from any signs of blight.

The ease with which artificial infections may be produced on the flowering quince, especially on the blossoms, is quite interesting. No effort whatever was made in these inoculation experiments, either in the sprays or in the injections, to prevent drying out of the inoculum, the inoculated parts being permitted to dry out naturally in the field and in the greenhouse. In spite of this, over 85 per cent of the inoculations were successful, and there is no reason to doubt that this percentage would have been greater had an effort been made to keep the twigs in a saturated atmosphere. In view of these facts why have no natural infections been found on this host and is there any good reason for believing that it is not subject to infection under the usual conditions? The only thing known to the writers that may conceivably interfere with blossom infection in this host is the earliness of its blooming period, but even this does not appear as a valid objection because some of the pear varieties, such as the Kieffer, bloom about the same time in the neighborhood of Fayetteville, Ark. On the other hand, there are excellent reasons for believing that where there is fire-blight inoculum which may be distributed to flowering quince blossoms (and bees, flies, ants, aphids, and other insects appear commonly in and around these), infections are apt to occur. It is quite possible that infections have been overlooked in the past because of the striking resemblance of blossom blight to frost injury and because of the fact that twig as well as blossom infections are inconspicuous, involving very little tissue. However this may be, there are very good indications that the disease may not be expected to do serious injury to the twigs of this host and that the blossoms are the only parts that suffer seriously.

ARTIFICIAL INFECTIONS ON THE ROSE

In 1925 Waite (*25*) succeeded in artificially infecting mature winter apples and rose cuttings by placing them under "forced conditions of the damp chamber, or bell jar." He placed three sets of three rose cuttings, whose lower ends were immersed in water, under bell jars lined with moistened filter paper. On the slanting surfaces of the free ends of two of these sets he smeared pure cultures of *Bacillus amylovorus*, while the third set was used as a control. The cuttings, of an unnamed variety, were in a "semidormant condition with the leaves slightly started into growth." They "somewhat reluctantly and somewhat feebly it is true, but nevertheless definitely, developed * * * blight" on the inoculated surfaces. Waite notes that before the rose cuttings developed very well marked cases of blight molds began to creep over the surfaces, putting an end to the experiment. In view of these facts it seemed desirable to ascertain the action of the fire-blight organism on roses without placing the inoculated parts under such "forced" conditions."

Good-sized twigs bearing flower buds were cut from a Fairfax rose which was making rapid growth out of doors. These cuttings were placed with their cut ends in water and inoculated by means of needle punctures as well as by injections with a hypodermic needle. Others, serving as checks, were wounded similarly and kept on a greenhouse bench close to the inoculated ones. No effort was made to prevent the inoculum from drying out or to keep the inoculated parts in an atmosphere surcharged with water. On April 10 some

15 inoculations were made, some on the newly developed twigs, others on the pedicels of the blossoms, and the remaining ones in the young ovaries. By April 13, three days after the inoculations were made, a number showed clear signs of blight in the form of darkened, almost blackish discolorations and a withering of the attacked parts. By the fifth day all of the inoculations showed blackish areas around the inoculated points extending in a number of cases for several inches beyond the centers of infection. (See

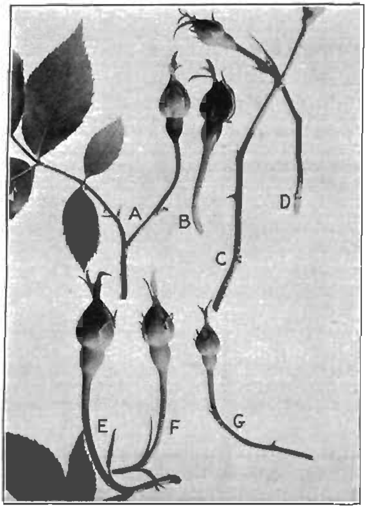

FIG. 3.—Artificial infections on twigs and flower buds of Fairfax rose: A, B, C, and D represent successful artificial infections; E, F, and G served as controls. Note the blackish discolored tissues of C and D, the discolored floral bud B, and the infected pedicel, A. Photographed four days after inoculating

Fig. 3.) All the checks remained healthy and there were no signs of discoloration or collapsing of tissues around the wounds. The disease appeared equally severe on the twigs, pedicels, and ovaries. In the last-named the disease producer invaded almost the whole flower bud, discoloring and killing the whole of the calyx, often including the tips of the lobes and a considerable part of the petals. Some of these blighted buds were placed in an ice chest and they developed typical oozing of the germs within 48 hours (fig. 4), the drops of ooze studding a large part of the infected areas. From one of the diseased buds the pathogene was recovered in pure culture and its virulence established by injecting it into healthy pear shoots and producing typical blight.

As only a limited amount of growth was obtained in these cut twigs subsequent to their excision, the blight would not be expected to travel for any considerable distance beyond the inoculation centers, and in no case did it extend more than about 4 inches. But, even assuming that the blighting would have been more extensive had the inoculations been made on unsevered, vigorously growing shoots, there were no indications in these artificial infections that the disease is capable of producing any severe infections on the plant as a whole. Nevertheless in this host, as in the flowering quince, the ease with which infections may be produced suggest the possibility of natural

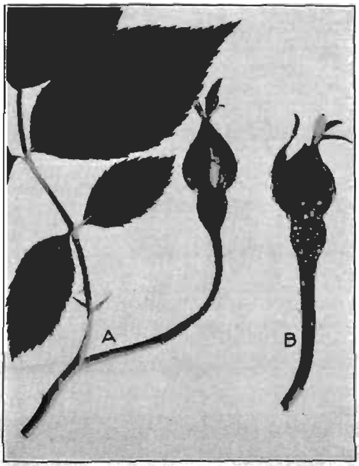

FIG. 4.—A and B, Artificial infections on rose showing large quantities of bacterial ooze, which appeared as globular, whitish drops on the calyxes, receptacles, and pedicels. Photographed six days after the inoculations were made

infections occurring readily and being overlooked because of their insignificant size and the slight damage that they occasion.

ARTIFICIAL INFECTIONS ON SPIREA

Having successfully produced infections on two different ornamental plants belonging to the rose family, it appeared worth while to attempt infections on the very common Spirea, *Spiraea vanhouttei* Zabel. As far as the writers know there are no records of the disease having been reported on this or other species of Spiraea in spite of the fact that the species noted above, as well as others, constitute some of the most frequently used ornamental plants in America. Unfortu-

nately a severe frost injured almost all of the bloom as well as some of the tender shoots, ruining incidentally the first attempts at artificial infection that had been made out of doors. Because the blossoms were not available, efforts were then confined to vigorously growing leafy shoots. A number of twigs were severed from a plant and placed with their cut ends in water, the twigs after inoculation being kept in the greenhouse. The method of inoculation was exactly the same as that used on Japanese quince and on rose, and resulted in typical blight (fig. 5) of 12 Spirea twigs within five days after inoculation. All control twigs remained healthy. The disease on this host differs somewhat in early symptoms from those previously given. The attacked parts which in most of the twigs involved several inches rendered the tissues extremely flaccid, in addition to the customary discoloration. But the odd effect was the appearance of the leaves attached to the diseased parts of the twig, which remained greenish and otherwise healthy looking for a considerable number of days after the twigs had contracted the disease. Eventually they succumbed, becoming brownish and gradually withering and dying completely. In the first few days after infection, however, the disease only involved the twigs and the lowermost parts of the leaf petioles, giving the leaf tissues the appearance of extreme resistance to the invading organism. Whether this is a normal reaction or whether it is merely due to the abeyance of host growth, and hence a lessening in susceptibility because of the twigs being severed, remains to be determined. It should be pointed out, however, that while the writers' artificial twig infections of pears have almost always led to a subsequent infection of subtending leaves, resulting in bacterial penetration of parts of the midrib and adjoining tissues, this was not the case in the Spirea infections. The subsequent death of the leaves may be considered as a secondary effect resulting from the killing of the adjoining twig portions. From one of the diseased twigs the organism was recovered and its pathogenicity established by inoculating pear twigs.

The general appearance of the disease in Spiraea, as in flowering quince, is markedly similar to the injury caused by frost. When diseased material is placed beside material injured by frost it is next to impossible to distinguish one from the other, and considering the fact that the early growth of Spiraea frequently occurs at a time of late spring frosts, at least in this section of the country, it is quite conceivable that the disease may occur naturally on this host without being detected. Inasmuch as no oozing of bacteria was obtained in any of the artificial infections, the disease on this plant is all the more apt to be confused with frost injury.

NATURAL INFECTION ON BURBANK PLUM

A number of drupaceous plants, mostly plum and cherries, have at times been reported as susceptible to *Bacillus amylovorus*. The first definite proof of susceptibility of a species of Prunus was advanced by L. R. Jones (*16*), who in 1902 with the assistance of L. P. Sprague, cultured the pathogene from blighted Cheney plum, *P. nigra* Ait. (*P. americana* var. *nigra*. Waugh), and produced typical infections on green pear fruits, a growing pear seedling, and on green plums, later recovering the organism from these artificial infections. M. B. Waite, according to Jones and to Smith (*22*,

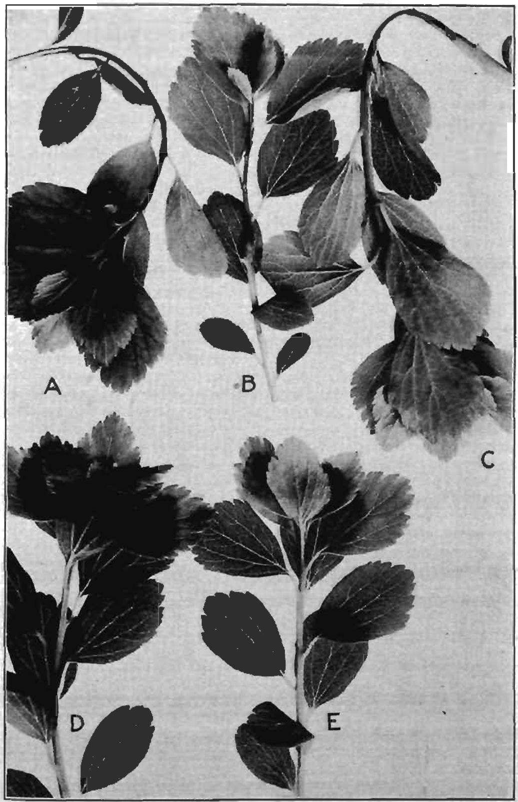

FIG. 5.—Artificial infections with *Bacillus amylovorus* on *Spiraea vanhouttei*: A, B, and C, Shoots inoculated with pure cultures by means of a hypodermic needle; D and E controls injected with sterile water. Photographed five days after inoculation

p. 359), also cultured the organism from blighted plums, but the varietal name is not given. Jones also reported that Waugh had observed the disease on hortulana plum, *P. hortulana* Bailey. In 1903 Paddock (*19*) showed that the blight on apricots was due to *B. amylovorus* and without giving proof he suggested that the blight on the apricot plum, *P. simonii* Carr, was due to the same organism. Whetzel (*27*) reported in 1909 "what appeared to be this disease killing prune trees" and Jackson (*15*) in 1915 fully substantiated this suggestion. The only other reference that has been found to plum or plumlike fruits which involved the fire-blight disease is that by Hotson (*14*) in 1916 who noted and figured blighted twigs on the yakimine, a cross between a prune and a peach, but he made no effort to obtain pure cultures and produce artificial infection.

It appears from the literature just cited that no one has noted the disease on any of the varieties of Japanese plum, *Prunus salicina* Lindl. (*P. triflora* Roxb. ex Bailey). It is therefore of interest to note that the Burbank plum, one of the varieties of the Japanese plum, was found by the senior writer to be blighted in May, 1928, in the region around Fayetteville, Ark. The disease was attacking twigs and leaf clusters of a single tree that was growing close to some badly blighted apple and pear trees. The damage was very slight, and in this respect is similar to that noted by other investigators of this disease on stone fruits in general. The organism was found within the attacked tissues and pure cultures were obtained. When grown on various culture media it appeared very similar to other strains obtained from apple and pear and when it was inoculated on vigorously growing pear shoots in the greenhouse it produced typical blight. From one of these blighted pear shoots the pathogene was recovered and from its cultural reactions was readily identified as typical *Bacillus amylovorus*. While the observations noted above indicate quite definitely that the disease is of minor importance on Burbank plum, it is necessary to bear in mind that there still is the possibility that the organism may be carried over winter in such hosts and serve as inoculating centers for susceptible host plants.

THE RANGE OF HOSTS OF BACILLUS AMYLOVORUS

The fact that new hosts have been added from time to time and that some of them, including very recent additions, have been published in journals not primarily devoted to research suggests the desirability of bringing them together in a list for ready consultation. It should be noted that a relatively large number of plants which have been reported by various individuals as subject to this disease are excluded from the list because the evidence is lacking. Among others, peach, almond, red raspberry, and blackberry, listed by Hewitt (*12*), are not included. No review of the literature concerning hosts will here be attempted in view of the excellent summary presented by Snow (*23*) in 1922. It may be worth while, however, to give the reasons for presenting some of the data in the list. There are very good reasons for believing that the disease has been recognized on some pomaceous plants since the latter part of the eighteenth century, as Arthur (*1*) first pointed out; but considering the fact that the true cause of the disease remained unknown until Burrill's time in 1878 (*7*), it becomes difficult to assign authorities

for some of the hosts. There can be little doubt that if one adheres rigidly to Koch's rules of proof (*17*) even Burrill's name would be excluded, since it is very questionable whether he worked with pure cultures. Nevertheless a reasonable consideration of his pioneer work on this disease must include his name as the authority for some of the hosts.

LIST OF PLANTS [a] SUSCEPTIBLE TO BACILLUS AMYLOVORUS

Scientific name	Common name	Investigators	Date
Amelanchier canadensis	Service berry	Arthur (*1*)	1885
Chaenomeles lagenaria (*Cydonia japonica*).	Flowering quince	Rosen and Groves	1928
Crataegus crusgalli	Cockspur thorn	Reed (*20*)	1914
Crataegus oxycantha	English hawthorn	Arthur (*1*)	1885
Crataegus oxycantha var. *splendens*	Double scarlet variety of English hawthorn.	Edwards (*9*)	1907
Crataegus pyracantha (see *Pyracantha coccinea*).			
Cydonia oblonga (*C. vulgaris*)	Quince	Burrill (7)	1881
Eriobotrya japonica	Loquat	Waite (*24*)	1907
Fragaria spp	Strawberry (wild and cultivated varieties).	Munn (*18*)	1918
Heteromeles arbutifolia	Tollon (Christmas berry)	Waite (*24*)	1907
Mespilus sp. [b]	Medlar	Waters (*8, 26*)	1921, 1922
Prunus armeniaca	Apricot	Paddock (*19*)	1903
Prunus avium	Royal Ann cherry and Bing cherry varieties.	Hotson (*15*)	1915
Prunus domestica	Prune	Whetzel, in Whetzel and Stewart (*27*), and Jackson (*15*).	1909, 1915
Prunus hortulana [b]	Hortulana plum	Waugh, in Jones (*16*).	1902
Prunus nigra (*P. americana* var. *nigra*).	Cheney plum	Jones (*16*)	1902
Prunus simonii [b]	Apricot plum	Paddock (*19*)	1903
Prunus triloba var. *plena*	Flowering almond	Snow (*23*)	1922
Pyracantha coccinea	Common fire thorn or evergreen thorn.	Arthur (*1*)	1885
Pyrus amygdaliformis	Chinese wild pear	Reimer (*21*)	1925
Pyrus baccata (or one of its hybrids)	Siberian crab (common crab)	Arthur (*2*)	1885
Pyrus balansae	Chinese wild pear	Reimer (*21*)	
Pyrus betulaefolia	do	do	1925
Pyrus bretschneideri	do	do	1925
Pyrus calleryana	Chinese pear	do	1925
Pyrus calleryana-dimorphophylla	Chinese wild pear	do	1925
Pyrus canescens	do	do	1925
Pyrus communis	Cultivated (European) pear	Burrill (7)	1881
Pyrus cordata	Chinese wild pear	Reimer (*21*)	1925
Pyrus coronaria	Wild garland crab	Arthur (*4*)	1887
Pyrus cotinifolia	Chinese wild pear	Reimer (*21*)	1925
Pyrus elaeagrifolia	do	do	1925
Pyrus fascicularis	do	do	1925
Pyrus faurieri	do	do	1925
Pyrus glabra	do	do	1925
Pyrus heterophylla	do	do	1925
Pyrus hondoensis	do	do	1925
Pyrus koehnei	do	do	1925
Pyrus longipes	do	do	1925
Purus malifolia	do	do	1925
Pyrus malus	Cultivated apple	Burrill (7)	1881
Pyrus mamorensis		Reimer (*21*)	1925
Pyrus michauxii		do	1925
Pyrus nivalis		do	1925
Pyrus ovoidea (hybrid?)		do	1925
Pyrus parviflora		do	1925
Pyrus paschia	Chinese wild pear	do	1925
Pyrus persica	do	do	1925
Pyrus phaeocarpa	do	do	1925
Pyrus salicifolia	do	do	1925
Pyrus serotina	Oriental pear	do	1925
Pyrus serrulata (hybrid?)	Chinese wild pear	do	1925
Pyrus sinaica	do	do	1925
Pyrus ussuriensis	Oriental pear	do	1925
Rosa sp	Unnamed cultivated variety and Fairfax rose.	Waite (*25*) and Rosen and Groves.	1925, 1928
Sorbus americana	American mountain ash	Burrill (7)	1881
Sorbus aucuparia var. *laciniata*	European mountain ash	Edwards (*9*)	1907
Spiraea vanhouttei	Vanhoutte spirea	Rosen and Groves	1928

[a] The scientific names used are those given by Bailey (*5*) except for the various oriental pear species, for which Reimer (*21*) is accepted as the guide.

[b] Complete evidence still wanting.

SUMMARY

A knowledge of the host range of *Bacillus amylovorus* is not only important from the standpoint of possible control measures but it also makes possible the study of parasitism in an exceptional bacterial species, one which, in spite of its wide host range, is able to penetrate into at least one kind of natural opening in numerous though diverse species and genera of rosaceous plants.

Three new hosts are here presented and confirmation afforded of another. They are, respectively, the common Japanese or flowering quince, the Vanhoutte spirea, the Burbank plum, and a cultivated rose of the Fairfax variety.

The Japanese quince, *Chaenomeles lagenaria*, was found to be susceptible in artificial infection experiments on blossoms and twigs, the pathogene being able to infect the blossoms very readily through the nectaries. No natural infections on this host have as yet been found and attention is called to the fact that the gross symptoms of the disease are very much like frost injury.

Artificial infections are readily accomplished on young twigs of Vanhoutte spirea, *Spiraea vanhouttei*, with symptoms comparable to frost injury on this host.

Natural infections have been found on the Burbank plum, *Prunus salicina*, from which the parasite was obtained in pure culture and was shown to be infectious on pear shoots.

Blossoms and young twigs of Fairfax rose are here described as being very susceptible to *Bacillus amylovorus* in artificial infections.

A list of all the known species of host plants is presented.

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